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Computational modeling and analysis of gene regulatory interaction network for metabolic disorder: a bioinformatics approach

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ABSTRACT

In this study, we generate a PPI network and co-regulatory networks to understand the mechanisms of metabolic disorder more clearly. This study also analyzes the relevance of genes that are responsible for Cardiovascular (CVD), Obesity (OBS), Type 2 diabetes (T2D) and Hypertension (HT). It also showed the common genome among CVD, OBS, T2D, and HT. Using Bioinformatics approaches, drugs are possible to design. For this study gene was collected from NCBI (National Center for Biotechnology Information) using R language. Primarily, 7197 genes were found for CVD, 3140 are for OBS, 3283 genes were for T2D and 2237 are for HT which were responsible for all species. Among those genes, 12 top-weighted common genes were selected for this research. Using these liable common genes, a protein-protein interaction network (PPI) and a regulatory interaction network were constructed. The PPI network shows the interaction among those genes. And the regulatory interaction network defines the direct and indirect connection among selected genes. The PPI network will help to design more reliable drug targets.

Keywords: *PPI network; Cardiovascular; Obesity; Type 2 diabetes; Hypertension; Bioinformatics; Drug design.*

1. INTRODUCTION

Worldwide, one main health problem is cardiovascular disease [1]. Gradually, it is being epi-demic. Worldwide Cardiovascular diseases are increasing day by day. In fact, at the age is greater than or equal 65, about 70% of type 2 diabetes mellitus died from CVD [1]. In their study, Patricia Rarau observed that high or low socioeconomic status group members are at risk of CVD. The mortality rate of CVD related disease more than three quarters occur in low and middle-income countries. Tobacco smoking, high blood pressure, high total and low-density lipoprotein (LDL) cholesterol, type 2 diabetes mellitus (T2DM) and physical inactivity are the primary risk factors for CVD. At least three of the five risk factors of CVD are associated with metabolic syndrome, which is related to the development of both CVDs and T2DM [2]. In 2012, worldwide 17500000 (31%) death occurred for CVD [3]. Globally, the morbidity and mortality rates for adults are increasing due to CVD [4]. Among women, 8.6 million deaths a year due to CVD, which construct one third of all deaths among women around the world [5]. Worldwide, 30% of all deaths are due to cardiovascular diseases [16].

The World Health Organization (WHO) has announced that obesity among adults is the larg-est chronic health problem in the world [26]. Overweight and obesity are the as abnormal or excessive fat collection, which is a risk to health and it is defined by WHO. The US Centers for Disease Control and Prevention (CDC) and the WHO defined the normal BMI range for adults are 18.5 to 24.9, while a BMI $\geq$ 25 kg/m^2 is deliberated to be overweight, and BMI $\geq$ 30 kg/m^2 is considered as obese, with severe obesity considered as a BMI $\geq$ 40 kg/m^2 [6]. In 2015, a total 1.9 billion and 609 million adults were calculated to be overweight and obese, which represents about 39% of the world's population [6]. The BMI is the appropriate method of measurement of the level of underweight and overweight, which accepted by WHO in 1995. When BMI is less than 21 kg/m2 than the risk of diabetes and HT will be the lowest [7]. It occurs when a person eats calories more than his need. Because of the extra calories are converted to fat. According to WHO (World Health Organization), in 2016, 39% men and women were overweight or obese worldwide and those who were eighteen or more. Overweight represents the main health burden, and it is associated with metabolic diseases, for example, insulin resistance and cardiovascular disease [8]. One of the main reasons for premature mortality is childhood obesity [9]. Worldwide up to 57.8% people (3.3 billion people) could be either overweight or obese if the ongoing propensities proceed by 2030 [10].

Type 2 diabetes mellitus (DM) is a complex metabolic disease which concurrent with insulin resistance and beta-cell debilitation leads to hyperglycemia, which is the sign of the malady [1]. It has two main types, one is type-1 diabetes and another is type-2 diabetes. Type-2 diabetes occur when the body unable to use the produced insulin effectively [13]. Globally, the DM Outbreak is increased which expectation is up to 42% from 2003 to 2025 and the prevalence rate of DM is moderately higher in females than in males whose age is less than 60 years [4]. Studies have demonstrated that over-weight can enhance the risk of T2DM [11]. In Asia, Diabetes was more dominant, which was increased by weight gain [7]. Worldwide, in 2017 about 425 million adults had diabetes and this number is anticipated to ascend to 629 million by 2045 [12].

Worldwide, the prevalence of Hypertension has increased extensively. Worldwide, Hypertension is a public health threat due

to its high frequency and concomitant risks of cardiovascular diseases [16]. Different physiological and behavioral risk factors like high body mass index, harmful use of alcohol, tobacco use, lead exposure, and a high-salt diet are the causes of hypertension and it is one of the CVD [17]. According to 2017, the American College of Cardiology (ACC) and the American Heart Association (AHA), ≥130 mm Hg and ≥80 mm Hg (millimeters of mercury) was the systolic blood pressure (SBP) and the diastolic blood pressure (DBP) respectively [18]. The increment of hypertension among the Bangladeshi adults whose ages is greater than or equal to 25 years to be 20% in 2010 [14], 21% in 2013 [15] and 17.5 % in 2015 [18]. Worldwide in 2000, the burden of hypertension was more than a quarter, which about one billion, in 2010, the burden was approximately 1.4 billion and It could exceed 1.6 billion by 2025 [16,19]. An effective relationship establishes among respiratory impairment and the presence of cardiovascular disease, diabetes mellitus and hypertension [20]. The risk factor of diabetes and cardiovascular diseases are related to metabolic syndrome [21]. Abnormalities in blood-pressure, lipid, lipoprotein, and insulin levels in adults, also the risk of coronary artery disease and diabetes are directly related to Childhood obesity [22]. Cardiovascular diseases are the main reasons for death in people with diabetes, and numerous components, including hypertension, contribute to an increase of the prevalence of CVD [23]. In observational and genetic studies, there is abundant evidence that type 2 diabetes mellitus related to the increased risk of major atherosclerosis-associated cardiovascular disease [24]. Obesity increases the risk of diabetes mellitus and cardiovascular disease [6]. Obesity is associated with type 2 diabetes and type 2 diabetes associated with the increased risk of cardiovascular [25]. A good relationship established between obesity and hypertension for both adults and children [26].

A metabolic syndrome is a group of symptoms that considered to obesity, type 2 diabetes mellitus, cardiovascular disease, stroke and insulin resistance, dyslipidemia and hypertension [27]. Metabolic syndromes in several ways are known as insulin resistance, abdominal obesity, insulin resistance, hypertension, and hyperlipidemia which are defined by WHO [28]. According to the above discussion, there is an interrelation among CVD, OBS, T2D and HT. And it is clear that CVD, OB, T2D, and HT directly or indirectly associated with the metabolic disorder syndrome.

2. MATERIALS AND METHODS

For creating a Protein-protein interaction network, some steps are performed individually. Those steps are Gene collection, Gene-filtering, Gene Cross linkage, Gene mining, PPI network. Each of the steps is described individually in the sub-section of 3.1 to 3.6 respectively.

2.1. Data source.

Genes are collected from NCBI (National Center for Biotechnology Information) using the R language which is responsible for CVD, OBS, T2D, and HT. NCBI is a vast and trusted gene database. It gives the opportunity to download the gene. On the other hand, R is better to analyze statistical data and graphics.

2.2. Gene filtering.

In the step of data source, genes are collected for CVD, OBS, T2D, and HT which are responsible for all sapiens. But in this step, genes are collected which are responsible only for humans. This study analyzes the human gene only. So, the collected Genes need to filter and only those genes are responsible for human diseases that are selected for the next step. This filtering is completed using the R language.

2.3. Gene sorting using R.

Four diseases genes are downloaded from NCBI using R by increasing order their weight. And the downloaded genes are stored in a text file by the using sorting technique of R.

2.4. Gene cross linkage.

This section will explore the relationships between the selected diseases. First, the linkage between the two diseases-genes is shown such as CVD and OBS genes; CVD and T2D genes; CVD and HT genes; OBS and T2D genes; OBS and HT genes; T2D and HT genes. And secondly, the linkage is shown among the three diseases- genes those are CVD, OBS, and HT genes; CVD, OBS and T2D genes; OBS, T2D and HT genes; CVD, T2D and HT genes. All the associated genes are collected in a text file.

2.5. Gene mining.

In this step, genes are mining using the R data mining technique. Gene mining is a valuable section of this study. Without gene mining, important data can miss. Without important data, the analysis will be wrong and the result gives the wrong output. So gene mining is very important to find the appropriate result.

2.6. Common gene collection.

Those genes are common among CVD, OBS, T2D and HT are collected using R. Top 100 and 50 weighted genes are collected for common gene collection.

2.7. PPI network.

Protein-protein Interaction (PPI) networks give numerous new insights into protein function. The PPI network gives a rich framework for a better understanding of the functional organization of the proteome [29]. There are many online tools to design the PPI network. Among them, UniHi is an online tool that is used to visualizing and analyzing the biological network.

3. RESULTS AND DISCUSSIONS

The main of this study is to design a PPI network and a Regulatory interaction network. In this step, findings are described respectively in the sub-section.

3.1. Gene collection, filtering & gene sorting.

In this study, 7197 genes for CVD, 3140 genes for OBS, 3283 genes for T2D and 2237 genes for HT are found primarily for all sapiens without filtering. After filtering, 970 genes for CVD, 1883 for OBS, 1824 for T2D and 1012 for HT are found only for *Homo sapiens*. All the genes are collected according to their weight and store them in a text file. Figure 1 shows the bar-diagram of collected genes for *Homo sapiens*. In Table 1, all genes of selected 4 diseases are exhibited.

3.2. Gene cross linkage.

The cross linkage occurred among selected diseases CVD, OBS, T2D and HT. Firstly, the linkage between the two disease genes has been linked. And secondly, the three disease genes have

been linked. Collected genes for two diseases are 577 for CVD & OBS, 728 for CVD & T2D, 615 for CVD & HT, 1086 for OBS & T2D, 610 for OBS & HT and 828 for T2D and HT. The genes 408 for CVD & OBS, 516 for CVD & T2D, 395 for CVD & HT, 646 for OBS & T2D, 376 for OBS & HT and 499 for T2D and HT are found only for Homo sapiens. Collected genes for three diseases are 334 for "CVD, OBS & HT", 431 for "CVD, OBS & T2D", 450 for "OBS, T2D & HT" and 421 for "CVD, T2D & HT". The genes 230 for "CVD, OBS & HT", 317 for "CVD, OBS & T2D", 290 for "OBS, T2D & HT" and 294 for "CVD, T2D & HT" are found only for Homo sapiens. Figures 2 and 3 represent the bar-graph of cross linkage of selected 2 and 3 diseases. In Table 2, all genes of cross linkages of selected 4 diseases are exhibited.

3.3. Gene mining and common gene finding.

285 genes are common among CVD, OBS, T2D and HT for all sapiens and 206 genes are common among CVD, OBS, T2D and HT only for Homo sapiens. Top 100 and 50 weight genes are selected and downloaded for creating PPI network. 12 genes are found which are top 100 and 50 weighted. And those genes are "TNF", "APOE", "VEGFA", "IL6", "MTHFR", "TGFB1", "ESR1", "ACE", "IL10", "HIF1A", "APP", and "MMP9". The genes are collected using the data mining techniques of R.

3.4. PPI network and regulatory interaction.

A PPI and regulatory interaction networks are created by using UniHi tool. Both networks show the direct and indirect connection among selected disease genes. Gene regulatory networks (GRNs) play a cardinal role in the cellular metabolism process in the increase of living organisms [30]. GRNs regulate all aspects of advancement, from cell fate specification to differentiation, by regulating the transcriptional evolution of transcription factors, molecular signaling, and all other genes involved in animal and all other genes that are involved in the formation of animals and plants [31]. Figure 4 shows the PPI network with 12 common genes and figure 5 shows the regulatory interaction network with 12 common genes. It is sure that 9 genes are interconnected which shown in figure 4 and figure 5 respectively. Those interconnection genes are TNF, ESR1, MMP9, IL6, IL10, HIF1A, APP, TGFB1 and VEGFA. Figures 6 and 7 have shown PPI network and regulatory interaction network respectively with those 9 interconnection genes. Figure 7 only shows those interacted proteins which are associated with selected disease genes.

The STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) is a biological database that predicts direct and indirect association of protein-protein interactions. Using STRING with 9 genes a network is created in figure 8.

GeneMania is a web-based Bioinformatics tool that predicts the functional gene sets. It shows the relation to a set of input genes. And it also shows the Co-expression, Co-localization, Physical Interactions, pathway, Genetics Interaction, and etc. A network is created using GeneMania that shown in figure 9.

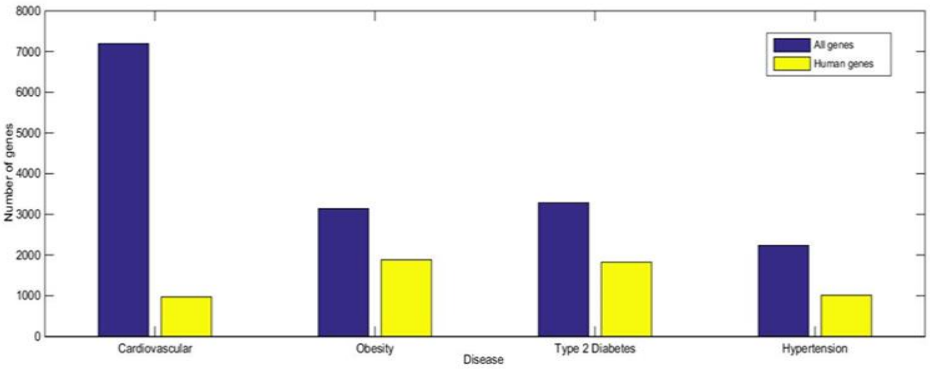

Figure 1. Gene collection bar-graph of all sapiens and *Homo sapiens* for selected diseases.

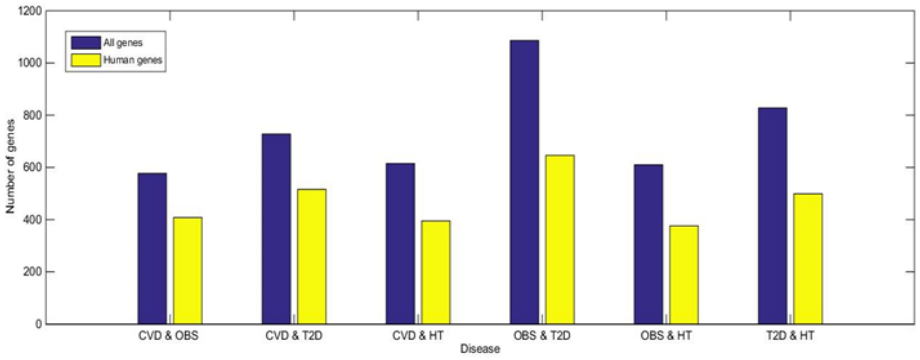

Figure 2. Bar-graph of cross-linkage of two disease genes.

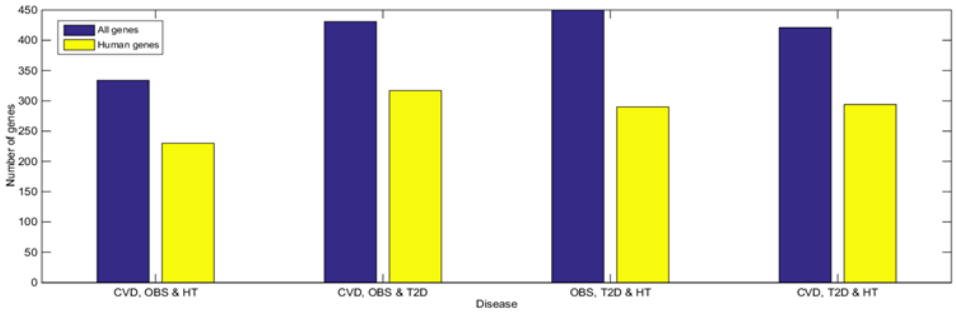

Figure 3. Bar-graph of cross-linkage of three disease genes.

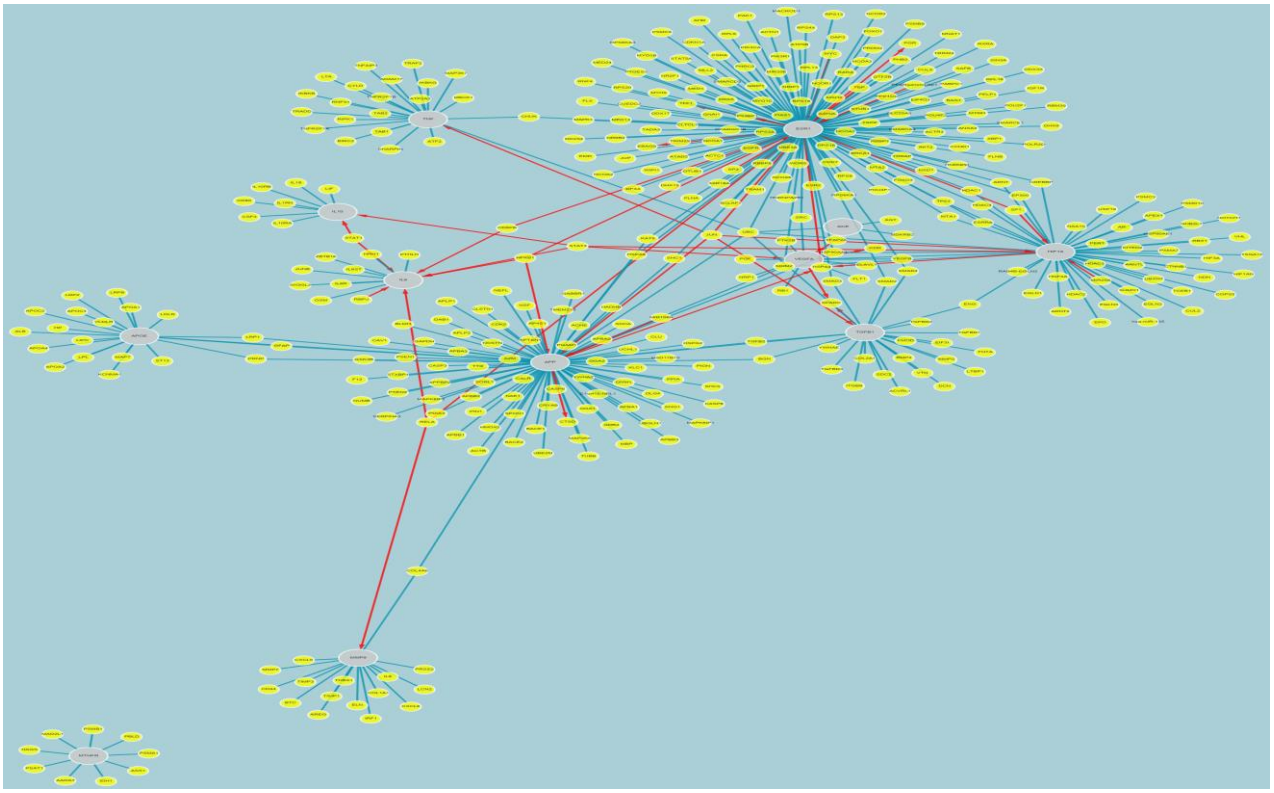

Figure 4. Protein-Protein Interaction Network with 12 common genes using UniHi tool.

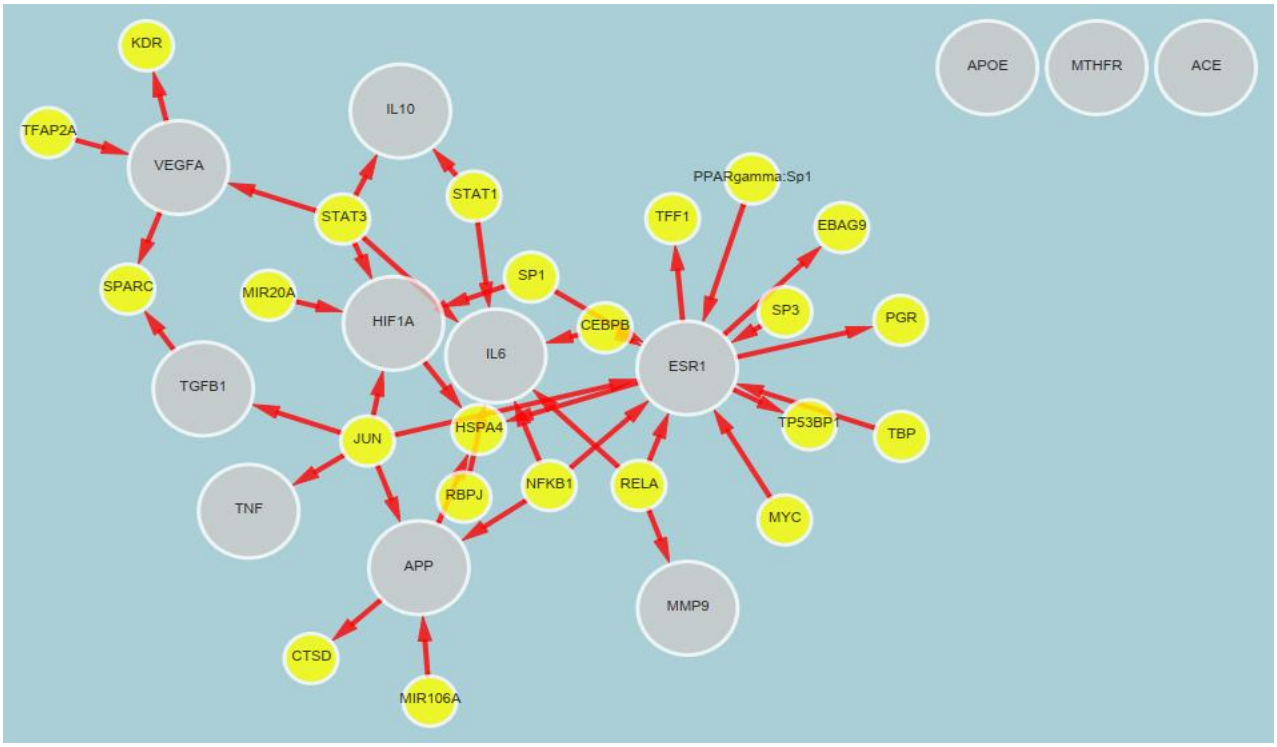

Figure 5. Regulatory Interaction Network with 12 common genes using UniHi tool.

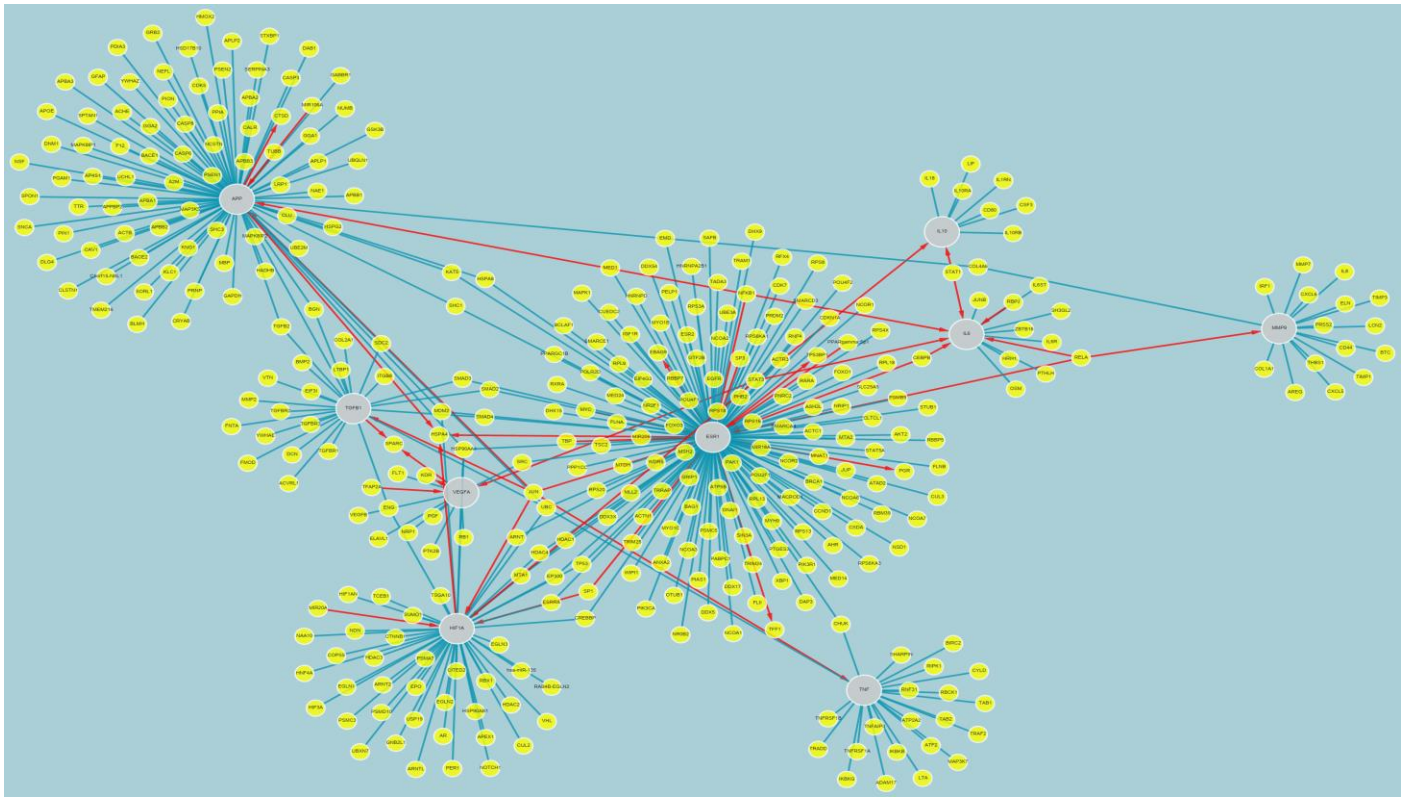

Figure 6. PPI network with 9 interconnected genes using UniHi tool.

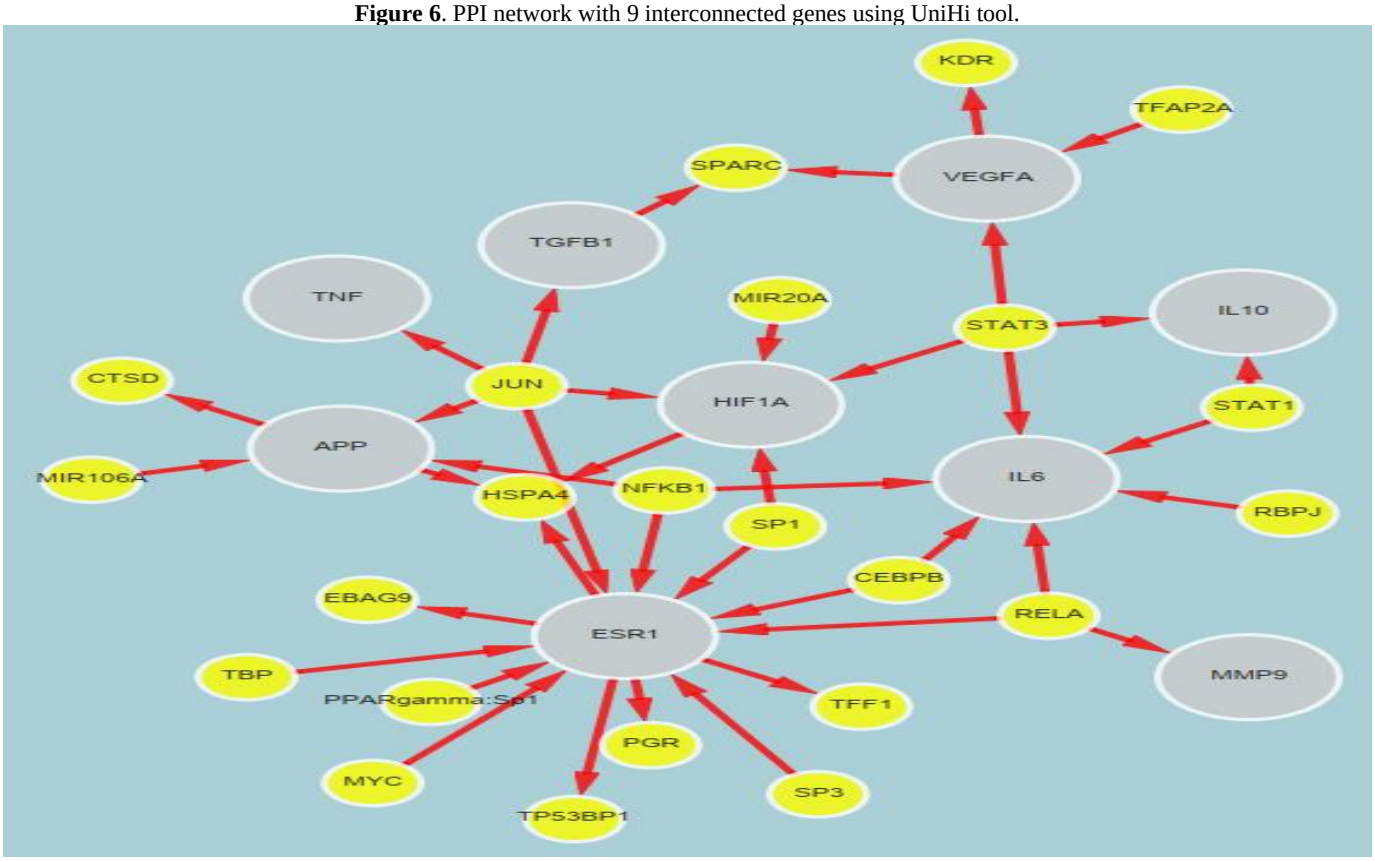

Figure 7. Regulatory interaction network with 9 genes.

Table 1. Gene collection table of all sapiens and Homo sapiens for four selected diseases.

Name of Diseases	Primary Number of Gene	Gene for only Human
Cardiovascular	7197	970
Obesity	3140	1883
Type2 Diabetes	3283	1824
Hypertension	2237	1012

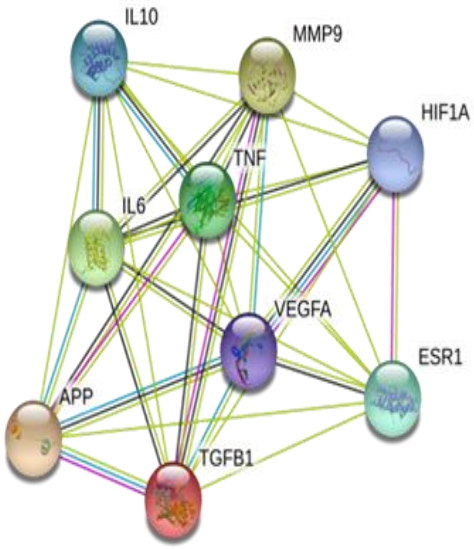

Figure 8. Network with 9 common genes using STRING.

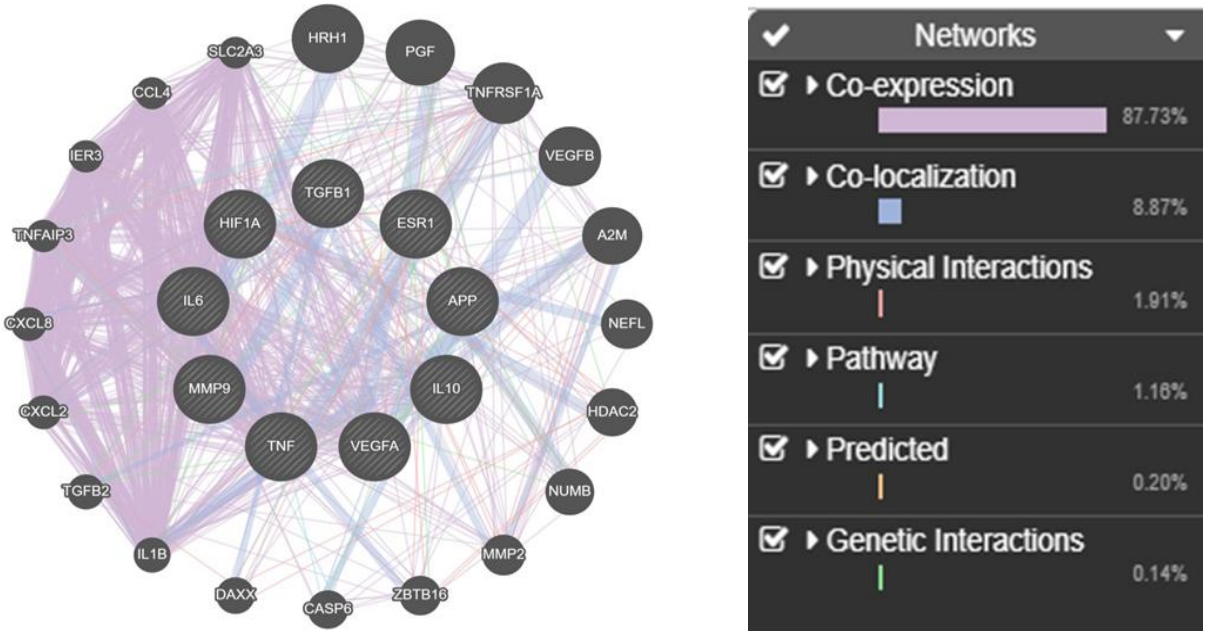

Figure 9. Network with 9 genes using GeneMania.

Table 2. Gene cross-linkage table for all sapiens and human among four selected Diseases.

Cross Linkage Between	No of Gene	
	All	Human
CVD and OBS	577	408
CVD and T2D	728	516
CVD and HT	615	395
OBS and T2D	1086	646
OBS and HT	610	376
T2D and HT	828	499
CVD, OBS and HT	334	230
CVD, OBS and T2D	431	317
OBS, T2D and HT	450	290
CVD, T2D and HT	421	294
CVD, OBS, T2D and HT	285	206

4. CONCLUSIONS

A metabolic disorder is a group of diseases that include CVD, OBS, T2D, HT, etc. Every day many people are dead because of metabolic disorders. After analyzing diseases and their affected genes, a drug can be designed using Bioinformatics knowledge and tools. PPI network and Regulatory Interaction Network are helpful to design a drug perfectly that can remedy the disease. This study analyzes four diseases and their associated genes. Top weighted genes are selected after processing and filtering, which are responsible for human. Using the common genes, the Regulatory Interaction Network and PPI network are created. Based on this study in our next project we will work on drug discovery.

5. REFERENCES

1. De Rosa, S.; Arcidiacono, B.; Chiefari, E.; Brunetti, A.; Indolfi, C.; Foti, D.P. Type 2 Diabetes Mellitus and Cardiovascular Disease: Genetic and Epigenetic Links. *Front Endocrinol (Lausanne)* **2018**, *9*, https://doi.org/10.3389/fendo.2018.00002
2. Rarau, P.; Pulford, J.; Gouda, H.; Phuanukoonon, S.; Bullen, C.; Scragg, R.; Pham, B.N.; McPake, B.; Oldenburg, B. Socio-economic status and behavioural and cardiovascular risk factors in Papua New Guinea: A cross-sectional survey. *PLOS ONE* **2019**, *14*, https://doi.org/10.1371/journal.pone.0211068
3. Pryzbek, M.; MacDonald, M.; Stratford, P.; McQuarrie, A.; Richardson, J.; McKelvie, R.; Tang, A. Long-term Enrollment in Cardiac Rehabilitation Benefits Cardiorespiratory Fitness and Skeletal Muscle Strength in Men With Cardiovascular Disease. *Canadian Journal of Cardiology* **2019**, *35*, 1359-1365 https://doi.org/10.1016/j.cjca.2019.05.018
4. Gutierrez, J.; Aladeen, A.; Mohammad, M.; Mohammad, A. Cardiovascular disease risk factors: Hypertension, diabetes mellitus and obesity among Tabuk Citizens in Saudi Arabia. *The open cardiovascular medicine journal* **2018**, *12*, https://doi.org/10.2174/1874192401812010041.
5. Sujane, K.; Anand. S.S. Cardiovascular disease among women from vulnerable populations: a review. *Canadian Journal of Cardiology* **2018**, *34*, 450-457, https://doi.org/10.1016/j.cjca.2018.01.017.
6. Chooi, Y.C.; Cherlyn, D.; Faidon, M. The epidemiology of obesity. Metabolism **2019,** *92*, 6-10, https://doi.org/10.1016/j.metabol.2018.09.005.
7. James, W.P.T. The epidemiology of obesity: the size of the problem. *Journal of internal medicine* **2008**, *263*, 336-352, https://doi.org/10.1111/j.1365-2796.2008.01922.x.
8. Zhang, X.M.; Guo, L.; Chi, M.-H.; Sun, H.M.; Chen, X.W. Identification of active miRNA and transcription factor regulatory pathways in human obesity-related inflammation. *BMC Bioinformatics* **2015**, *16*, https://doi.org/10.1186/s12859-015-0512-5
9. Arora, T.; Grey, I. Chapter 31 - Sleep, obesity and cardiometabolic disease in children and adolescents. In: *Sleep and Health.* Grandner, M.A. Ed. Academic Press: 2019; pp. 421-433, https://doi.org/10.1016/B978-0-12-815373-4.00031-9.
10. Kelly, T.; Yang, W.; Chen, C.S.; Reynolds, K.; He, J. Global burden of obesity in 2005 and projections to 2030. *International Journal of Obesity* **2008**, *32*, 1431-1437, https://doi.org/10.1038/ijo.2008.102.
11. Sjöblad, S. Could the high consumption of high glycaemic index carbohydrates and sugars, associated with the nutritional transition to the Western type of diet, be the common cause of the obesity epidemic and the worldwide increasing incidences of Type 1 and Type 2 diabetes? *Medical Hypotheses* **2019**, *125*, 41-50, https://doi.org/10.1016/j.mehy.2019.02.027.
12. Gomes, M.B.; Rathmann, W.; Charbonnel, B.; Khunti, K.; Kosiborod, M.; Nicolucci, A.; Pocock, S.J.; Shestakova, M.V.; Shimomura, I.; Tang, F.; Watada, H.; Chen, H.; Cid-Ruzafa, J.; Fenici, P.; Hammar, N.; Surmont, F.; Ji, L. Treatment of type 2 diabetes mellitus worldwide: Baseline patient characteristics in the global DISCOVER study. *Diabetes Research and Clinical Practice* **2019**, *151*, 20-32, https://doi.org/10.1016/j.diabres.2019.03.024
13. Goldenberg, R.; Punthakee, Z. Definition, Classification and Diagnosis of Diabetes, Prediabetes and Metabolic Syndrome. *Canadian Journal of Diabetes* **2013**, *37*, S8-S11, https://doi.org/10.1016/j.jcjd.2013.01.011
14. Rahman, M.; Zaman, M.M.; Islam, J.Y.; Chowdhury, J.; Ahsan, H.A.M.N.; Rahman, R.; Hassan, M.; Hossain, Z.; Alam, B.; Yasmin, R. Prevalence, treatment patterns, and risk factors of hypertension and pre-hypertension among Bangladeshi adults. *Journal of Human Hypertension* **2018**, *32*, 334-348, https://doi.org/10.1038/s41371-017-0018-x
15. Zaman, M.M.; Bhuiyan, M.R.; Karim, M.N.; MoniruzZaman; Rahman, M.M.; Akanda, A.W.; Fernando, T. Clustering of non-communicable diseases risk factors in Bangladeshi adults: An analysis of STEPS survey 2013. *BMC Public Health* **2015**, *15*, 659, https://doi.org/10.1186/s12889-015-1938-4.
16. Kearney, P.M.; Whelton, M.; Reynolds, K.; Muntner, P.; Whelton, P.K.; He, J. Global burden of hypertension: analysis of worldwide data. *The Lancet* **2005**, *365*, 217-223, https://doi.org/10.1016/S0140-6736(05)17741-1
17. Meiqari, L.; Essink, D.; Wright, P.; Scheele, F. Prevalence of Hypertension in Vietnam: A Systematic Review and Meta-Analysis. *Asia Pacific Journal of Public Health* **2019**, *31*, 101-112, https://doi.org/10.1177/1010539518824810.
18. Islam, J.Y.; Zaman, M.M.; Haq, S.A.; Ahmed, S.; Al- Quadir, Z. Epidemiology of hypertension among Bangladeshi adults using the 2017 ACC/AHA Hypertension Clinical Practice Guidelines and Joint National Committee 7 Guidelines. *Journal of Human Hypertension* **2018**, *32*, 668-680, https://doi.org/10.1038/s41371-018-0087-5.
19. Egan, B.M.; Kjeldsen, S.E.; Grassi, G.; Esler, M.; Mancia, G. The global burden of hypertension exceeds 1.4 billion people: should a systolic blood pressure target below 130 become the universal standard? *Journal of Hypertension* **2019**, *37*, 1148-1153, https://doi.org/10.1097/HJH.0000000000002021.
20. Mannino, D.M.; Thorn, D.; Swensen, A.; Holguin, F. Prevalence and outcomes of diabetes, hypertension and cardiovascular disease in COPD. *European Respiratory Journal* **2008**, *32*, 962-969, https://doi.org/10.1183/09031936.00012408.
21. Eckel, R.H.; Grundy, S.M.; Zimmet, P.Z. The metabolic syndrome. *Lancet* **2005**, *365*, 1415-1428, https://doi.org/10.1016/s0140-6736(05)66378-7.
22. Rocchini, A.P. Childhood obesity and a diabetes epidemic. *N. Engl. J. Med.* **2002**, *346*, 854-855, https://doi.org/10.1056/NEJM200203143461112.
23. Sowers, J.R.; Epstein, M.; Frohlich, E.D. Diabetes, hypertension, and cardiovascular disease: an update. *Hypertension* **2001**, *37*, 1053-1059, https://doi.org/10.1161/01.HYP.37.4.1053.
24. Larsson, S.C.; Wallin, A.; Håkansson, N.; Stackelberg, O.; Bäck, M.; Wolk, A. Type 1 and type 2 diabetes mellitus and incidence of seven cardiovascular diseases. *International Journal of Cardiology* **2018**, *262*, 66-70, https://doi.org/10.1016/j.ijcard.2018.03.099.

25. Ritchie, S.A.; Connell, J.M.C. The link between abdominal obesity, metabolic syndrome and cardiovascular disease. *Nutrition, Metabolism and Cardiovascular Diseases* **2007**, *17*, 319-326, https://doi.org/10.1016/j.numecd.2006.07.005
26. Michail, N.; Antza, C.; Doundoulakis, I.; Stabouli, S.; Kotsis, V. Hypertension in Obesity: Novel Insights. *Current Hypertension Reviews* **2020,** *16*, 30-36, https://doi.org/10.2174/1573402115666190415154603.
Björntorp, P.; Rosmond, R. The metabolic syndrome — a neuroendocrine disorder? *British Journal of Nutrition* **2000**, *83*, S49-S57, https://doi.org/10.1017/S0007114500000957.
27. Saklayen, M.G. The Global Epidemic of the Metabolic Syndrome. *Current Hypertension Reports* **2018**, *20*, 12, https://doi.org/10.1007/s11906-018-0812-z.
28. Stelzl, U.; Worm, U.; Lalowski, M.; Haenig, C.; Brembeck, F.H.; Goehler, H.; Stroedicke, M.; Zenkner, M.; Schoenherr, A.; Koeppen, S.; Timm, J.; Mintzlaff, S.; Abraham, C.; Bock, N.; Kietzman, S.; Goedde, A.; Toksoz, E.; Droege, A.; Kribitsch, S.; Korn, B.; Birchmeier, W.; Lehrach, H.; Wanker, E.E. A Human Protein-Protein Interaction Network: A Resource for Annotating the Proteome. *Cell* **2005**, *122*, 957-968, https://doi.org/10.1016/j.cell.2005.08.029.
29. Mishra, S.; Mishra, D. Enhanced gene ranking approaches using modified trace ratio algorithm for gene expression data. *Informatics in Medicine Unlocked* **2016**, *5*, 39-51, https://doi.org/10.1016/j.imu.2016.09.005.
30. Peter, I.S. Chapter 2-Methods for the experimental and computational analysis of gene regulatory networks in sea urchins. In: *Methods in Cell Biology*, Hamdoun, A.; Foltz, K.R.; Eds. Academic Press: Volume 151, 2019; pp. 89-113, https://doi.org/10.1016/bs.mcb.2018.10.003.

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