

## Article

# Identification of Key Signaling Pathways and Novel Computational Drug Target for Oral Cancer, Metabolic Disorders and Periodontal Disease

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**Abstract:** Oral cancer (OC), periodontal disease (PD), type 2 diabetes (T2D), and obesity are the most fatal disorders in the globe, generating frequent human issues. It is proposed in cutting-edge works that PD and OC are linked. T2D and obesity, on the other hand, are risk factors for PD, although the shared molecular mechanisms that underpin T2D, PD, OC, and obesity have yet to be identified. We intended to find common pathways and significant molecular biomarkers in PD, OC, T2D, and obesity to potentially clarify the connection between OC and PD and T2D and obese patients. Four Gene Expression Omnibus (GEO) microarray datasets (GSE29221, GSE15773, GSE16134, and GSE13601) are used for finding differentially expressed genes (DEGs) for T2D, obese, and PD patients with OC infection in order to explore comparable pathways and therapeutic medications. Gene ontology (GO) and pathway analyses were used to investigate the functional annotations of the genes. The hub genes were then identified using protein-protein interaction (PPI) networks, and the most significant PPI components were evaluated using a clustering approach. These three gene expression-based datasets yielded a total of seven common DEGs. According to the GO annotation, the majority of the DEGs were connected with the microtubule cytoskeleton structure involved in mitosis. The KEGG pathways revealed that the concordant DEGs are connected to the cell cycle and progesterone-mediated oocyte maturation. Based on topological analysis of the PPI network, We revealed major hub genes (CCNB1, BUB1, TTK, PLAT, and AHNAK) and notable modules. This work additionally identified the connection of TF genes and miRNAs with common DEGs, as well as TF activity. Predictive drug analysis yielded concordant drug compounds involved with T2D, OC, PD, and obesity disorder, which might be beneficial for examining the diagnosis, treatment, and prognosis of metabolic disorders and Oral cancer.

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## 1. Introduction

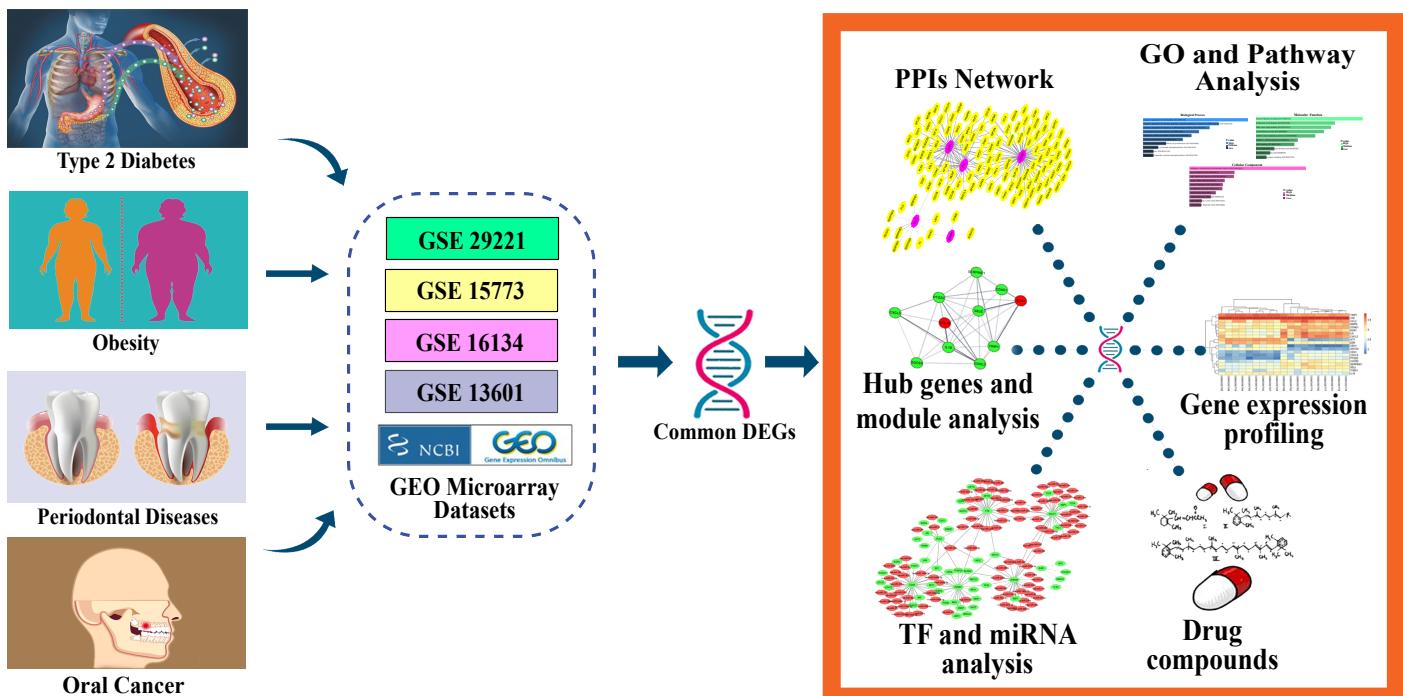
Metabolic syndrome is a diverse set of symptoms that are thought to originate from visceral adipose tissue obesity, hypertension, and improper glucose and cholesterol metabolism. Mitigating metabolic syndrome is of significant medical relevance because the

presence of several risk factors increases the chance of developing PD [1]. PD is a chronic phlogistic condition that reflects risk factors and conventional determinants with the key inflammatory disorders that cause almost two-thirds of all deaths, such as cardiovascular disease, cancer, diabetes, and severe respiratory disease [2]. Also, the incidence of OC has risen in recent years, especially among the young. Obesity, improper nutrition, and lack of physical exercise have been closely related to an increased risk of PD [3,4]. Trends in risk factors are anticipated to have an effect on the prevalence of PD, and obesity/diabetes disorder will further increase the incidence of PD [2]. Recently, the new categorization of periodontal conditions and disorders has acknowledged the significance of obesity. Moreover, obesity is considered one of the chronic metabolic disorders that damage the gingival attachment apparatus by regulating periodontal inflammation [5,6].

The first study on the relationship between being overweight and PD was reported in 1977, and it found that overweight rats are more susceptible than normal rats to have periodontal tissue degradation [7]. Obese Japanese individuals were shown to be more sensitive to PD than healthy people in 1998 [8]. In that experiment, a greater BMI was associated with a larger proportion of PD in 241 reportedly healthy individuals aged 20 to 59 years, who were assessed for overweight employing BMI and skin fat and gingival status using the community periodontal index (CPI). In a multiple-regression study, Buhlin et al. [9] also found that a greater BMI, as well as cholesterol, were frequently related to chronic PD. The buildup of visceral fat, which is connected with obesity, raises the risk of multiple adult illnesses, particularly T2D [10]. Nishimura et al. [11] discovered a link between BMI and PD in individuals who had T2D in another investigation. According to the findings of these investigations, obesity may be linked to PD in a form that is unrelated to diabetes.

As indicated earlier, obesity is the leading risk factor for T2D, and both T2D and obesity are growing more widespread and rapidly turning severe health issues [12]. Numerous studies have highlighted that PD is much more widespread in T2D and deteriorates with diabetes [13], and PD is often considered the sixth consequence of diabetes [14]. Biological treatment may be used as a specialized cancer treatment or to mitigate some adverse effects related to cancer, such as the deleterious consequences of chemotherapy [15]. Large-scale microarray datasets are required for assessing meaningful biological information based on gene expression [16]. The previous study provides evidence of the relationship among PD, OC, T2D, and obesity disorders [1, 17], but the analysis of large-scale microarray data for PD, OC, T2D, and obesity has not yet been elucidated.

In this work, four datasets were utilized to determine the biological connection between PD, OC, T2D, and obesity. These datasets were gathered from an online data repository that is known as the GEO database where GSE16134, GSE13601, GSE29221, and GSE15773 for PD, OC, T2D, and obesity, respectively. Details of the datasets are mentioned in table 1 and the overall workflow of the study is represented in figure 1. The primary work was to detect DEGs for GSE16134, GSE13601, GSE29221, and GSE15773 and then revealed concordant DEGs for PD, OC, T2D, and obesity. Here, the most important genes to experiment on for this complete research are the concordant DEGs. In order to get a better understanding of the biological processes that are uncovered by genome-based expression investigations, further experiments, and analyses were carried out using the common DEGs. These analyses included pathway analysis and enrichment analysis. Retrieving hub genes from concordant DEGs is a key part of selecting candidate drugs that depend mostly on hub genes. PPI networks are also constructed using commonly used DEGs to discover hub genes. Subsequently, similar DEGs are also used to analyze transcriptional regulatory agencies of GSE16134, GSE13601, GSE29221, and GSE15773. Finally, It is proposed that therapeutic medications be used. Because, when it requires to treat OC, there are not many solutions. It is critical to focus the efforts of research on the creation and refinement of novel treatment methods for OC. Conventional treatments for diagnosing this form of cancer, such as chemotherapy as well as surgery, have progressed substantially over the last thirty to forty years, but their adverse effects continue to cause patients anxiety [15].



**Figure 1.** The overall workflow of current investigation. Four datasets GSE16134, GSE29221, GSE15773, and GSE13601 are collected according to PD, T2D, obesity, and OC in human cells, respectively to conduct the study.

As a result, it is essential to research and employs less dangerous drugs for patients, as well as to create methods to lessen chemotherapy-induced negative effects, which prevent numerous therapeutic hazards. From this perspective, therapeutic drugs are proposed to avoid other negative effects [15]. The objective of this research is to identify novel biomarkers and molecular pathways that could be used as OC, PD, T2D, and obese diagnostic and therapeutic biomarkers.

## 2. Materials and Methods

### 2.1. Datasets

We used microarray datasets from the NCBI GEO collection to investigate common biological links among T2D, PD, OC, and obesity [18]. The GSE29221 dataset analyses gene expression data from Type 2 Diabetes patients. GPL6947 Illumina HumanHT-12 V3.0 expression bead chip platform is completed for GSE29221 to get microarray data analysis. Jain P et al. [19] provided the GSE29221 dataset. The GSE13601 dataset examines OC gene expression data. GSE13601 contains 58 samples, 27 of which are benign and 31 of which are malignant. The PD dataset has the GEO entry Code GSE16134 and contains 69 healthy samples and 241 periodontal human tissue impacted by PD. The GSE16134 dataset was sequenced using the GPL570 [HG-U133\_Plus\_2] high-throughput sequencing technology supplied by Papapanou PN et al. [20]. The obesity dataset, GSE15773, was derived from 9 subcutaneous adipose tissue and 10 omental adipose tissue. For the GSE15773 dataset, which is [HG-U133\_Plus\_2] Affymetrix Human Genome U133 Plus 2.0 Array [21], the GPL570 framework was used. Table 1 displays the datasets' summarised description.

### 2.2. Extraction of DEGs and concordant DEGs among OC, PD, T2D, and obesity

When there is a significant distinction between multiple test scenarios at the transcription level, a gene is said to be differentially expressed [22]. The principal goal of this study is to determine DEGs for the datasets GSE29221, GSE15773, GSE13601, and GSE16134. We selected those four microarray datasets because these datasets provide a greater number of DEGs. The DEGs were discovered using the R-programming language's (R-PL) limma

**Table 1.** Overview of datasets including their geo-properties and evaluation metrics

| Disorder name | GEO accession | GEO platform | Total DEGs | Up regulated DEGs | Down regulated DEGs |
|---------------|---------------|--------------|------------|-------------------|---------------------|
| OC            | GSE13601      | GPL8300      | 1658       | 668               | 990                 |
| T2D           | GSE29221      | GPL6947      | 721        | 111               | 610                 |
| PD            | GSE16134      | GPL570       | 2071       | 1083              | 988                 |
| Obesity       | GSE15773      | GPL570       | 480        | 174               | 306                 |

library. The DESeq2 [23] and limma package [24] are used in order to obtain the data that are generated from microarray analysis. In several testing scenarios, the false discovery rate was handled using the Benjamini-Hochberg method [25]. Cut-off criteria (adjusted P-value < 0.05 and  $|\log FC| \geq 1.0$ ) was considered for both GSE29221, GSE13601, and GSE15773. DEGs for the GSE16134 dataset were found using adjusted P-value between 0.5 and 1.5 and absolute  $\log FC \geq 1.0$ . The mutual DEGs of GSE29221, GSE15773, GSE13601, and GSE16134 were retrieved using the R-PL.

### 2.3. GO and pathway enrichment (PE) analysis

Gene set enrichment assessment explores gene sets with broad biological activities and chromosomal regions [26]. Gene ontology (GO) terms are utilized to annotate gene products. These concepts are classified as biological mechanisms, cellular components, and transcription factors [27]. The major motivation for the establishment of GO nomenclature is to better understand the molecular function, cellular function, and the location within a cell where genes perform their activities. KEGG pathway is often utilized for the comprehension of signaling pathways as well as has a substantial impact on gene annotation [28]. In addition to the KEGG pathway database, WikiPathways [29], Reactome [30], and BioCarta repositories were employed for substantial pathway analysis. A web-based platform Enrichr was used to retrieve GO terms and all of the pathways for the concordant genes that were found in the previous phase. For investigated genomic sequences Enrichr facilitates gene set enrichment experiments on online platforms.

### 2.4. Network analysis for PPIs

The function of PPIs is regarded as the primary goal of the biomedical study and aids as a prerequisite for network biology [31]. Proteins conduct their activity inside a cell by interacting with another protein, and the feedback supplied by a PPI network enhances understanding of the molecule's activity [32]. In order to generate a PPI network, Concordant DEGs are inserted into the Screening Toolbox for Associated Genes (STRING). The information that STRING yields is based on both experimental and expected interactions and the interaction that is created via the online tool is characterized by 3D frames, supplementary data, and a confidence score [33]. Additionally, the confidence measure was also employed for the existing PPIs structure, which was given a value of 0.400 on the moderate confidence spectrum. In order to establish a degree of confidence that falls somewhere in the middle the STRING tool was utilized. For a more accurate representation of the network and to specify top leading genes, Cytoscape (<https://cytoscape.org/>) is used to evaluate the retrieved PPIs. Cytoscape serves as the most effective program for integrating with bigger datasets of genetic connections, protein–protein interactions, as well as protein–DNA connections [34].

### 2.5. Hub gene extraction and module analysis

In a PPI structure, interactions are represented by edges between nodes, and the gene with the highest number of edges is called a hub gene. Cytoscape is used for the PPI network analysis in the present study. Hub proteins for the relevant PPIs structure are identified by employing the Cytoscape software plugin cytoHubba. The user-friendly interface of cytoHubba makes it the most significant hub identification plugin for Cytoscape,

as well as it offers 11 techniques for topological experiments [35]. The degree topological technique is used to determine the hub genes for the present study. The degree algorithm was chosen above other methods because it provides insight into the density of association between each gene in the PPIs network and because it suggests dense modules as the primary method for analyzing the PPIs. Hub genes generate highly enriched regions, which may be extracted as a significant component from the PPIs structure. In addition, the MCODE feature of the Cytoscape tool is utilized to identify the most substantial components inside the PPIs structure. Through MCODE clustering, extremely interrelated regions are discovered, which helps the study in generating more efficient drugs. MCODE is employed in the process of determining highly linked regions in order to represent molecular complexes in the PPIs structure [36].

### 2.6. TF-gene interactions (TF-GI)

TF-gene association with the discovered concordant DEGs is evaluated to determine the effect of TF on both functional pathways as well as interpretation profiles of genes [37]. The NetworkAnalyst tool is employed in order to determine TF-gene interchanges using previously discovered concordant genes. NetworkAnalyst is an integrated online platform that permits users to launch gene expression for a wide variety of species as well as provides them with the ability to do meta-analysis [38]. The ENCODE database which is a part of the NetworkAnalyst tool was employed to retrieve the network that was developed for the TF-GI network.

### 2.7. TF-miRNA coregulatory (TF-mC) network

Associations for TF-mC connections were obtained from a database known as the RegNetwork [39], which allows for the identification of miRNAs and governmental TFs that govern DEGs of relevance during transcription and post-transcription. NetworkAnalyst was used to study the TF-mC network. NetworkAnalyst aids scientists in traversing complicated information to uncover biological processes and functions, supporting the creation of relevant biological hypotheses [40].

### 2.8. Evaluation of candidate drugs

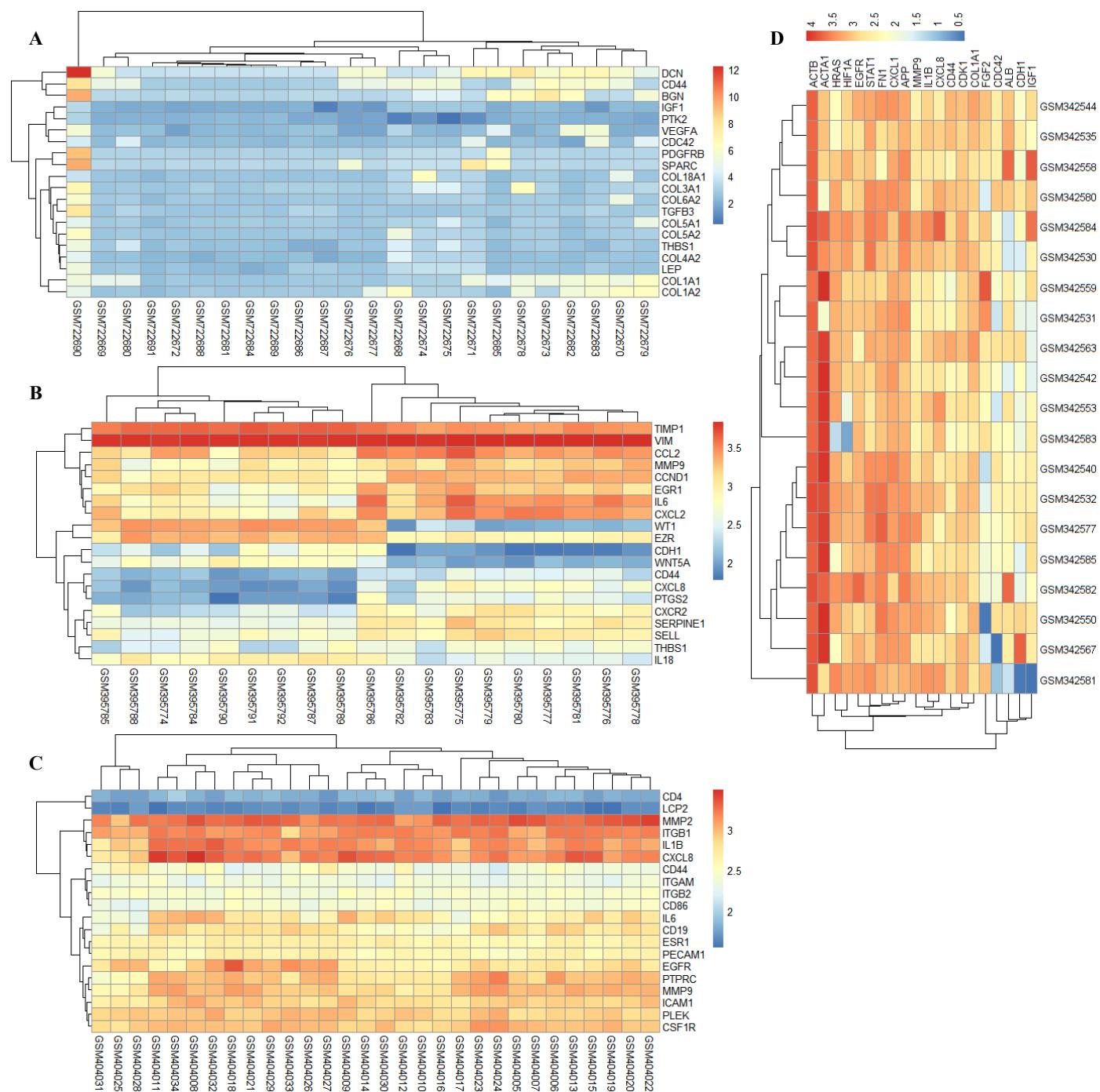
The designation of drug molecules is the primary focus of the current study. Utilizing the Drug Signatures database (DSigDB), which has 22527 gene sets, drug molecule is assembled using similar DEGs for T2D, PD, and obesity disorders. Enrichr (<https://amp.pharm.mssm.edu/>) is the platform that is used in order to get access to the DSigDB database. Enrichr is primarily employed as an enrichment evaluation tool that displays several graphical features on the collective activities of the input genes [41].

## 3. Results

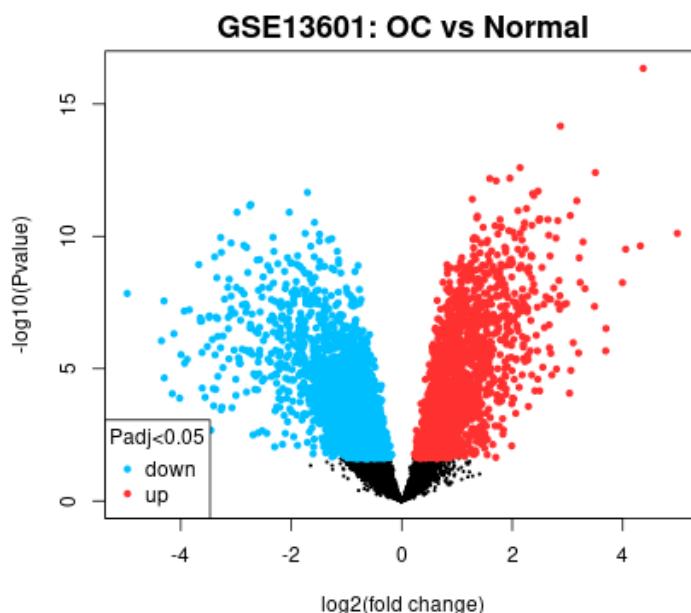
### 3.1. Gene expression (GE) analysis of OC, T2D, PD, and obesity

From the GSE29221 dataset, a total of 24 samples were examined and type 2 diabetes infection was discovered in those samples. In the field of system biology, infection refers to the process through which a foreign pathogen, such as a virus, fungi, bacteria, or other microbes, invades and colonizes an organism. Figure 2A depicts the GE of the top 20 genes from the samples taken, making it easier to concentrate on the significant expression profile of the DCN and BGN genes. In addition, characterization of gene expression is reported for all 19 samples of adipose tissue and healthy controls, which includes nine insulin-resistant patient samples and the other patient samples being insulin sensitive. The GE of the top 20 genes from the GSE15773 dataset is depicted in Figure 2B. Furthermore, the characterization of GE is described for 30 samples, including eight normal participants (GSM404031, GSM404025, GSM404028, GSM404010, GSM404016, GSM404017, GSM404007, and GSM404013), and the remaining 310 are PD samples. The difference between the PD observations and the healthy controls elucidates the various categories of PD observations, as shown in Figure 2C. The distinction between the OC data and the healthy controls

elucidates the numerous classifications of OC observations, as shown in Figure 2D. A volcano plot is shown, and the corrected P-value of 0.05 is used to show the upregulated and downregulated genes that have been detected. via a comparison of T2D cases and normal cases for the GSE29221 dataset (Figure 3).



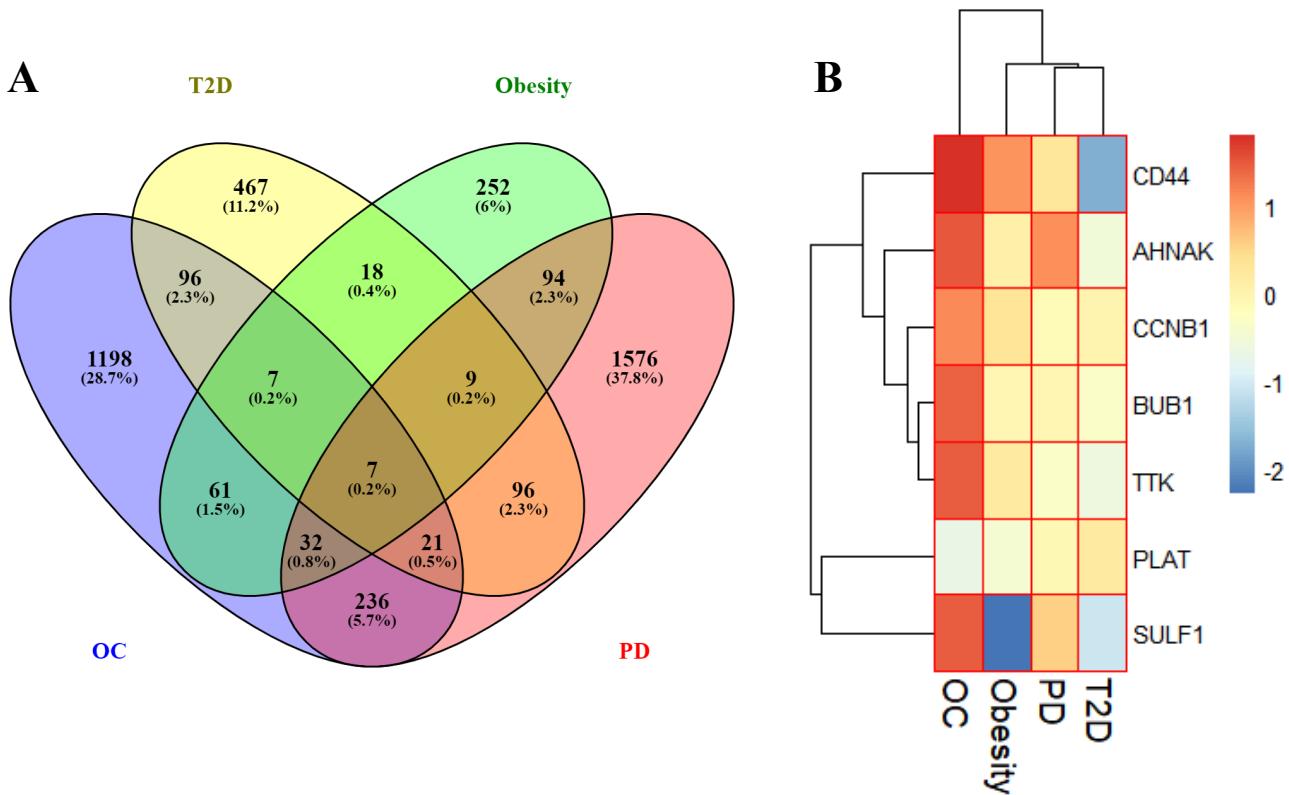
**Figure 2.** (A) Analyzing the GE of T2D disorder in human cells using the leading 20 genes and 24 samples from the GSE29221. (B) GE analysis and visualization of healthy controls and obesity-affected samples from the GSE15773. (C) GE patterns in gingival tissues of PD patients for the leading 20 genes and selected 30 samples from the GSE16134. (D) GE analysis and visualization of healthy controls and OC samples from the GSE13601.



**Figure 3.** The volcano plot displays the gene regulation (upregulated and downregulated) for the GSE13601 dataset.

### 3.2. Extraction of DEGs and concordant DEGs among OC, T2D, PD, and obesity

To explore the interconnections and effects of T2D, obesity, and PD with OC, we evaluated the dysregulated genes that successively enhance T2D, obesity, OC, and PD using the NCBI human microarray datasets. Experiments on the microarray dataset were performed in the R language environment, which included the limma and DESeq2 packages, and used the false discovery rate calculated by Benjamin-Hochberg. To begin, 721 genes were found to be differently expressed in T2D patients, along with 111 up-regulated and 610 down-regulated genes uncovered. Similarly, our assessment identified the most important DEGs for obesity and PD after conducting various statistical analysis processes. We found 2071 DEGs in the PD dataset (1083 up-regulated and 988 down-regulated) and 480 DEGs in the Obesity dataset (174 up-regulated and 306 down-regulated). For the OC dataset, a total of 1658 DEGs were analyzed, including 668 upregulated and 990 downregulated genes that met the criteria for assessment. All substantial genes, those are differentially expressed DEGs, are retrieved on the assumption of  $P\text{-value} < 0.05$  and  $|\log FC| \geq 1$ . After conducting a comparative evaluation on Jvenn, a renowned online platform for Venn analysis, we discovered 11 genes that are differentially expressed and shared by the T2D, obesity, and PD datasets. We use this concordant gene set to carry out an additional study. These four diseases are associated with each other because they share genes with one another [42]. The overall comparative assessment of the four datasets and the extraction of the common DEGs were shown in Figure 4A. The heatmap displaying the log fold change for genes that are the same across T2D, obesity, and PD demonstrated a significant transcriptional signature (Figure 4B).



**Figure 4.** (A) This investigation includes three microarray datasets for OC (GSE13601) T2D (GSE29221), PD (GSE16134), and Obesity (GSE15773). This comprehensive analysis demonstrated that OC, PD, obesity, and T2D share 7 common DEGs. (B) GHeat map based on the log fold changes for shared similar DEGs among T2D, Obesity, and PD datasets.

### 3.3. GO and PE analysis

Enrichr was employed to execute GO and PE analysis to identify the biological significance and enriched pathways revealed in this study utilizing common DEGs. GO incorporates gene features and their constituents into a concern to provide broad precise knowledge resources. Theoretically, ontology describes a corpus of knowledge within a certain environment. Both ontology and annotation are intended to produce a complete model of the biological structure that actually aids biological applications [43]. The GO repository served as the annotation channel for the study, which was conducted across three categories (biological feature (BF), molecular function (MF), and cellular component (CC)). Table 2 lists the top 10 terms in BF, MF, and CC. Figure 5 depicts a linear representation of the entire ontological assessment as bar graphs for each type.

Analysis of pathways demonstrates that the organism responds to its intrinsic changes. It is a hypothetical method for displaying how numerous disorders interact via fundamental molecular or biological processes [44]. The most significant pathways of DEGs shared by T2D, obesity, OC, and PD were determined from four universal databases, namely KEGG, WikiPathways, BioCarta, and Reactome. The top pathways extracted from the specified datasets are summarized in Table 3. To explain more appropriately, Figure 6 also depicted the PE analysis in the bar charts.

**Table 2.** GO terms, GO pathways, and the genes for shared DEGs with their associated P-values

| Category              | GO ID      | Term  | P-value     | Genes          |
|-----------------------|------------|---|-------------|----------------|
| GO biological process | GO:1902850 | microtubule cytoskeleton organization involved in mitosis                                       | 8.80E-06    | CCNB1;TTK;BUB1 |
|                       | GO:0007052 | mitotic spindle organization  | 1.62E-05    | CCNB1;TTK;BUB1 |
|                       | GO:0071173 | spindle assembly checkpoint signaling   | 2.20E-05    | TTK;BUB1       |
|                       | GO:0007094 | mitotic spindle assembly checkpoint signaling   | 2.20E-05    | TTK;BUB1       |
|                       | GO:0071174 | mitotic spindle checkpoint signaling  | 2.20E-05    | TTK;BUB1       |
|                       | GO:0045841 | negative regulation of mitotic metaphase/anaphase transition                                    | 2.42E-05    | TTK;BUB1       |
|                       | GO:0061448 | connective tissue development   | 7.34E-05    | SULF1;CD44     |
|                       | GO:0051216 | cartilage development   | 1.38E-04    | SULF1;CD44     |
|                       | GO:0090100 | positive regulation of transmembrane receptor protein serine/threonine kinase signaling pathway | 4.33E-04    | TTK;SULF1      |
| GO molecular function | GO:0001501 | skeletal system development   | 1.27E-03    | SULF1;CD44     |
|                       | GO:0005113 | patched binding   | 0.002447753 | CCNB1          |
|                       | GO:0061575 | cyclin-dependent protein serine/threonine kinase activator activity                             | 0.002797015 | CCNB1          |
|                       | GO:0005540 | hyaluronic acid binding   | 0.003844172 | CD44           |
|                       | GO:0004065 | arylsulfatase activity  | 0.004890388 | SULF1          |
|                       | GO:0008484 | sulfuric ester hydrolase activity   | 0.005935662 | SULF1          |
|                       | GO:0004712 | protein serine/threonine/tyrosine kinase activity   | 0.009760292 | TTK            |
|                       | GO:0043539 | protein serine/threonine kinase activator activity  | 0.012880151 | CCNB1          |
|                       | GO:0016538 | cyclin-dependent protein serine/threonine kinase regulator activity                             | 0.015300881 | CCNB1          |
| GO cellular component | GO:0004896 | cytokine receptor activity  | 0.030400721 | CD44           |
|                       | GO:0019887 | protein kinase regulator activity   | 0.033804687 | CCNB1          |
|                       | GO:0045121 | membrane raft   | 0.001349594 | AHNAK;SULF1    |
|                       | GO:0062023 | collagen-containing extracellular matrix  | 0.007098193 | PLAT;SULF1     |
|                       | GO:0005925 | focal adhesion  | 0.007353827 | AHNAK;CD44     |
|                       | GO:0030055 | cell-substrate junction   | 0.007613669 | AHNAK;CD44     |
|                       | GO:0000307 | cyclin-dependent protein kinase holoenzyme complex  | 0.010454324 | CCNB1          |
|                       | GO:1902554 | serine/threonine protein kinase complex   | 0.012880151 | CCNB1          |
|                       | GO:0044291 | cell-cell contact zone  | 0.016336778 | AHNAK          |
|                       | GO:0042383 | sarcolemma  | 0.018061198 | AHNAK          |
|                       | GO:0005902 | microvillus   | 0.019783027 | CD44           |
|                       | GO:0098858 | actin-based cell projection   | 0.028694887 | CD44           |

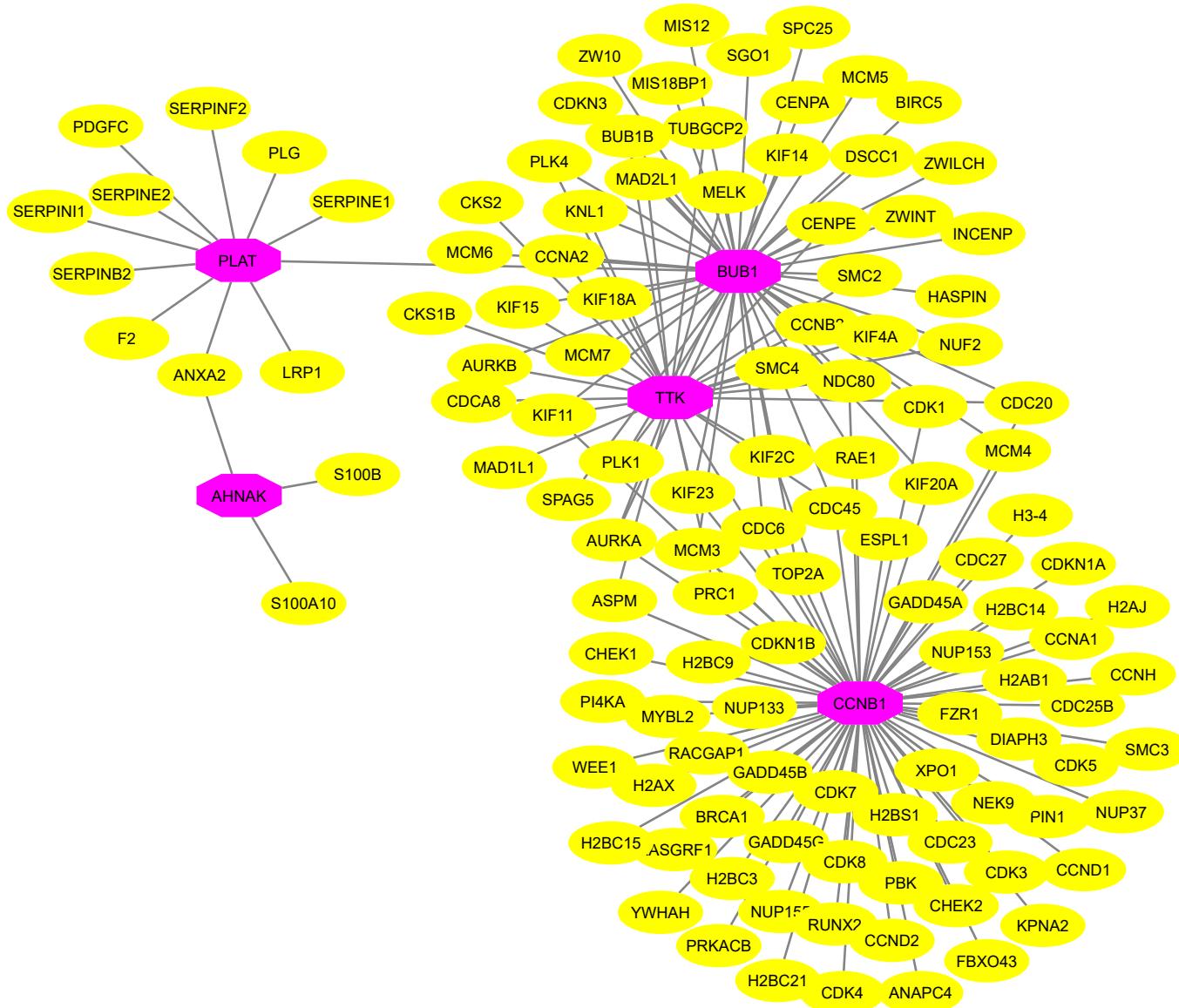
### 3.4. PPIs network construction to detect hub genes (HG) and module analysis

We analyzed the PPI network from STRING and depicted it in Cytoscape to determine the associations and adhesion mechanisms of concordant DEGs. Figure 7 illustrates the PPI network of concordant DEGs, which has 125 nodes and 161 edges. In a PPI network, the majority of associated nodes are recognized as hub genes. Based on the PPI network analysis using the Cytohubba plugin in Cytoscape, we recognized the top five DEGs in terms of their level of influence. The HG are namely CCNB1, BUB1, TTK, PLAT, and AHNAK. These hub genes have the potential to be used as biomarkers, which might eventually lead to the growth of novel therapy methods for the disorders being explored. As HGs are feasible, we have designed a submodule network (Figure 8) with the help of the Cytohubba plugin to better comprehend their associations and proximity. The extended network of the HG interconnections generated from the PPI network is displayed in Figure 8. Topological assessment for the HG (CCNB1, BUB1, TTK, PLAT, and AHNAK)

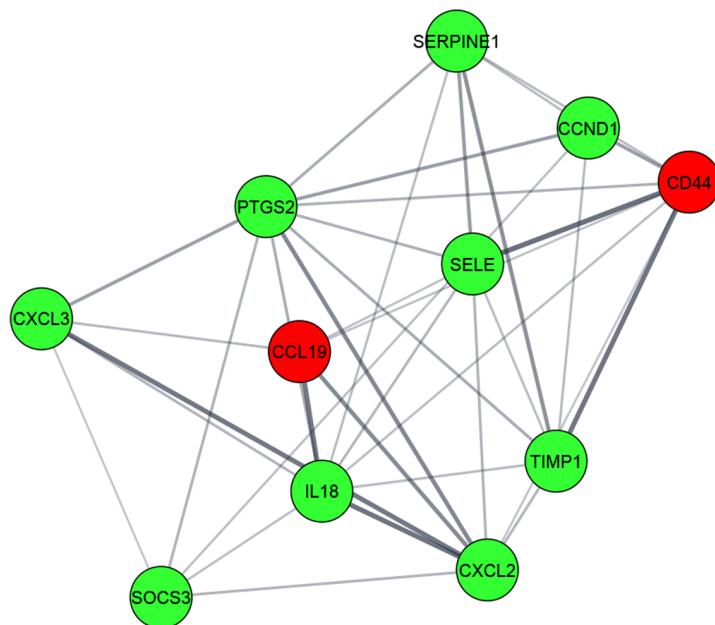
**Table 3.** Pathway enrichment analysis of similar DEGs among OC, T2D, Obesity, and PD

| Databases    | Pathways   | P-value     | Genes          |
|--------------|--|-------------|----------------|
| KEGG         | Cell cycle   | 7.99E-06    | CCNB1;TTK;BUB1 |
|              | Progesterone-mediated oocyte maturation  | 0.000511333 | CCNB1;BUB1     |
|              | Oocyte meiosis   | 0.000848724 | CCNB1;BUB1     |
|              | p53 signaling pathway  | 0.025275502 | CCNB1          |
|              | Complement and coagulation cascades  | 0.029377529 | PLAT           |
|              | ECM-receptor interaction   | 0.030400721 | CD44           |
|              | Prostate cancer  | 0.033464752 | PLAT           |
|              | Hematopoietic cell lineage   | 0.034144519 | CD44           |
|              | FoxO signaling pathway   | 0.044965206 | CCNB1          |
|              | Apelin signaling pathway   | 0.046982473 | PLAT           |
| WikiPathways | Cell cycle WP179   | 7.24E-06    | CCNB1;TTK;BUB1 |
|              | Retinoblastoma gene in cancer WP2446   | 0.000387282 | CCNB1;TTK      |
|              | Senescence and Autophagy in Cancer WP615   | 0.000563545 | PLAT;CD44      |
|              | Regulation of sister chromatid separation at the metaphase-anaphase transition WP4240                  | 0.005238917 | BUB1           |
|              | NOTCH1 regulation of endothelial cell calcification WP3413   | 0.005935662 | PLAT           |
|              | Blood Clotting Cascade WP272   | 0.00802339  | PLAT           |
|              | ATM Signaling Pathway WP2516   | 0.01391823  | CCNB1          |
|              | Fibrin Complement Receptor 3 Signaling Pathway WP4136  | 0.014264049 | PLAT           |
|              | ATM Signaling Network in Development and Disease WP3878  | 0.015646284 | BUB1           |
|              | Hepatitis C and Hepatocellular Carcinoma WP3646  | 0.017026857 | CD44           |
| Reactome     | Resolution Of Sister Chromatid Cohesion R-HSA-2500257  | 0.000574287 | CCNB1;BUB1     |
|              | G2/M DNA Replication Checkpoint R-HSA-69478  | 0.001748917 | CCNB1          |
|              | Mitotic Prometaphase R-HSA-68877   | 0.001751927 | CCNB1;BUB1     |
|              | Phosphorylation Of Emi1 R-HSA-176417   | 0.002098387 | CCNB1          |
|              | Activation Of NIMA Kinases NEK9, NEK6, NEK7 R-HSA-2980767  | 0.002447753 | CCNB1          |
|              | Insulin-like Growth Factor-2 mRNA Binding Proteins (IGF2BPs/IMPs/VICKZs) Bind RNA R-HSA-428359         | 0.002447753 | CD44           |
|              | Mitotic Anaphase R-HSA-68882   | 0.00270764  | CCNB1;BUB1     |
|              | Mitotic Metaphase And Anaphase R-HSA-2555396   | 0.002730626 | CCNB1;BUB1     |
|              | E2F-enabled Inhibition Of Pre-Replication Complex Formation R-HSA-113507                               | 0.003146172 | CCNB1          |
|              | Condensation Of Prometaphase Chromosomes R-HSA-2514853   | 0.003495224 | CCNB1          |
| BioCarta     | Sonic Hedgehog Receptor Ptc1 Regulates cell cycle Homo sapiens h ptc1Pathway                           | 0.003146172 | CCNB1          |
|              | AKAP95 role in mitosis and chromosome dynamics Homo sapiens h akap95Pathway                            | 0.004890388 | CCNB1          |
|              | Platelet Amyloid Precursor Protein Pathway Homo sapiens h plateletAppPathway                           | 0.004890388 | PLAT           |
|              | Estrogen-responsive protein Efp controls cell cycle and breast tumors growth Homo sapiens h EfpPathway | 0.005238917 | CCNB1          |
|              | Fibrinolysis Pathway Homo sapiens h fibrinolysisPathway  | 0.005238917 | PLAT           |
|              | Regulation of cell cycle progression by Plk3 Homo sapiens h plk3Pathway                                | 0.006283878 | CCNB1          |
|              | Cell Cycle: G2/M Checkpoint Homo sapiens h g2Pathway   | 0.007675696 | CCNB1          |
|              | How Progesterone Initiates the Oocyte Maturation Homo sapiens h mPRPathway                             | 0.00802339  | CCNB1          |
|              | Cyclins and Cell Cycle Regulation Homo sapiens h cellcyclePathway                                      | 0.00802339  | CCNB1          |
|              | Stathmin and breast cancer resistance to antimicrotubule agents Homo sapiens h stathminPathway         | 0.008370979 | CCNB1          |

is determined with the use of cytohubba. The findings of the topological assessment are shown in Table 4.



**Figure 5.** PPI network of common DEGs among OC, T2D, Obesity, and PD. The circular nodes in the diagram point to DEGs, while the edges recollect the relations between nodes. There are 91 nodes and 92 edges in the PPI network. The PPI network was formed using String and shown with Cytoscape.



**Figure 6.** Module analysis derived from Figure 7 PPIs network. CCL19 and CD44 are displayed in red color as these two leading genes are similar among GSE13601, GSE29221, GSE16134, and GSE15773. The network depicts heavily linked regions of the PPI network.

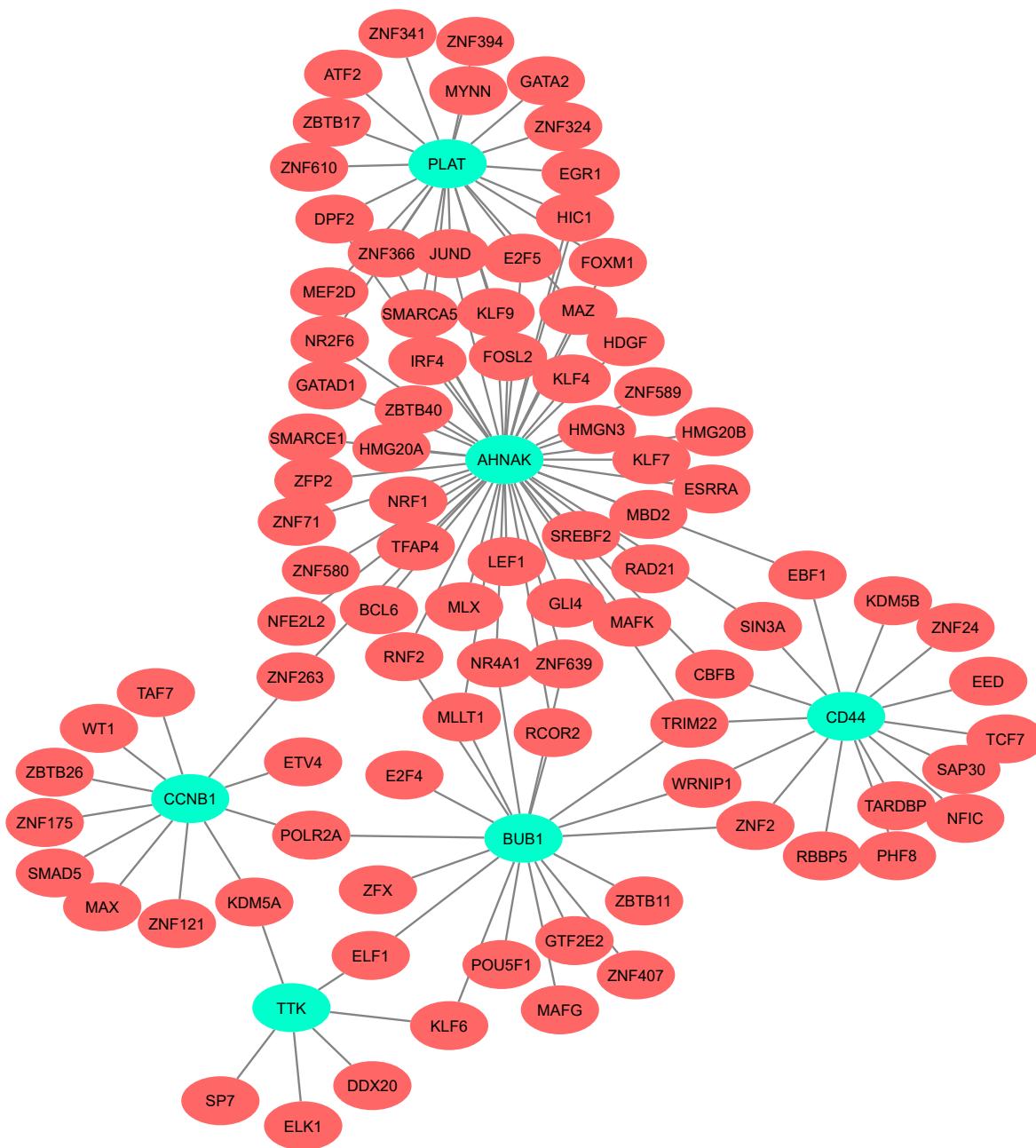
**Table 4.** Investigation of the top five hub genes based on topological results

| Hub gene | Degree | Stress | Closeness centrality | Betweenness centrality |
|----------|--------|--------|----------------------|------------------------|
| CCNB1    | 70     | 157116 | 0.556962             | 5.634944               |
| BUB1     | 47     | 158248 | 0.640625             | 5.031449               |
| TTK      | 32     | 26298  | 1.0                  | 1.314330               |
| PLAT     | 11     | 59543  | 0.916667             | 1.826842               |
| AHNAK    | 3      | 502    | 0.73333              | 1.333333               |

### 3.5. TF-gene interactions

NetworkAnalyst was used in order to compile the TF-gene interactions. For the concordant DEGs (CCNB1, BUB1, TTK, PLAT, CD44, SULF1, AHNAK) the TF-genes were detected. Figure 9 illustrates the interchange of TF regulators with similar DEGs. The network builds with 96 nodes and 120 edges. According to the interaction produced by the TF-gene network, CD44 is controlled by 15 TF genes, and CCNB1 is controlled by 11 TF genes. The network consists of 90 TF genes in total. These 90 TF genes are responsible for the regulation of more than one common DEG, which implies a high level of interchange between the TF genes and common DEGs. The TF-gene interaction network is illustrated in Figure 9.

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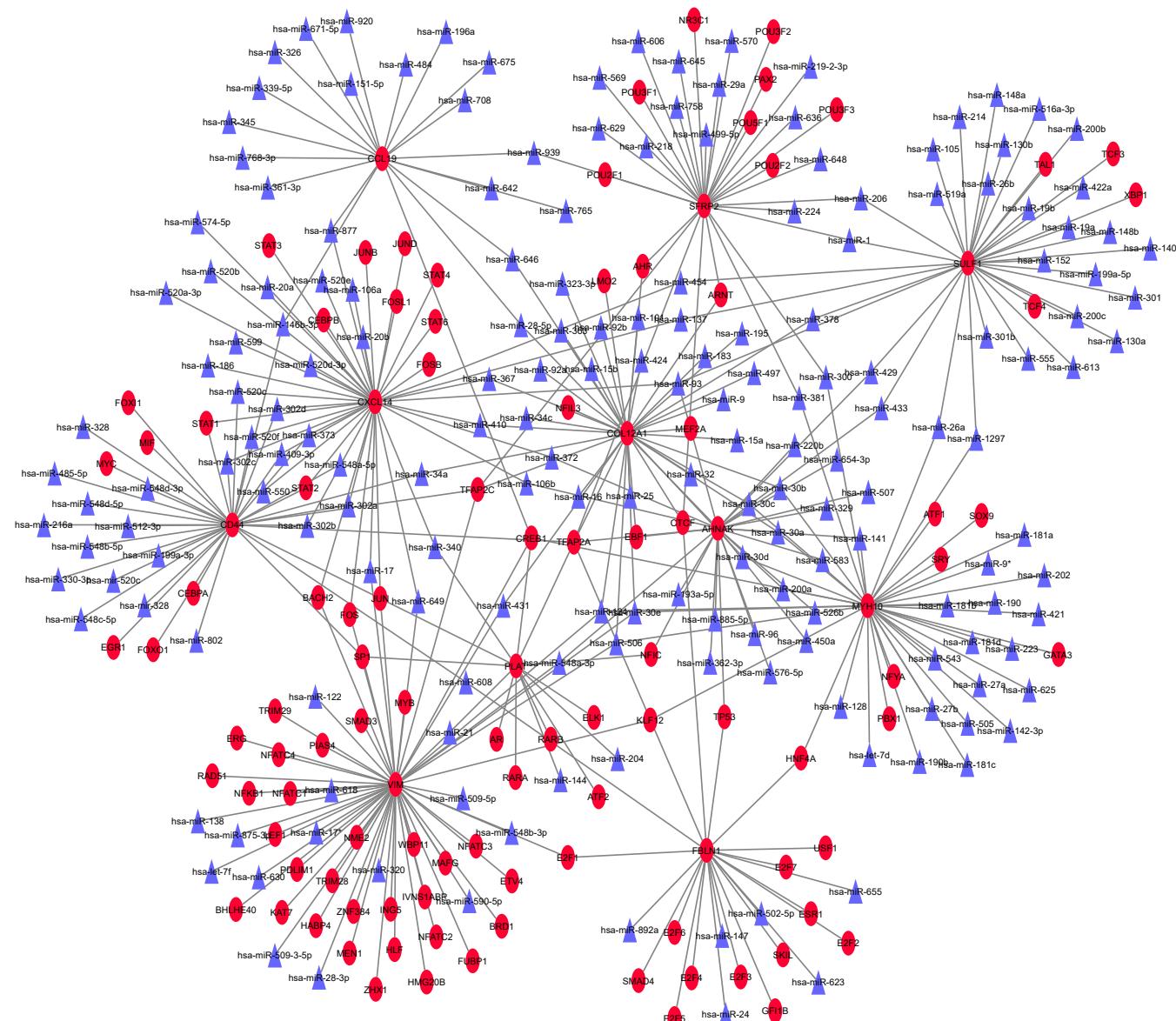


**Figure 7.** Interaction network for TF-genes with similar DEGs. The highlighted blue node denotes concordant genes, whereas the remaining nodes are TF genes. The network includes 154 nodes and 204 edges.

### 3.6. TF-mC network

TF-mC network is constructed with the help of NetworkAnalyst. The evaluation of the TF-miRNA regulatory network provides information on the interactions between miRNAs and TFs with the shared DEGs. This relation has the potential to be the factor that regulates the expression of the DEGs. There are 153 nodes and 172 edges in the TF-mC network. 57 TF genes and 115 miRNAs have correlated with the concordant DEGs. The TF-mC network is displayed in Figure 10.

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**Figure 8.** The network demonstrates the TF-mC network. The network has 296 nodes and 367 edges, containing 128 TF genes, and 238 miRNAs with the common DEGs. The nodes in blue color illustrate miRNA and other nodes point to TF-genes.

### *3.7. Extraction of potential drugs*

Evaluation protein-drug interactions are fundamental to knowing the structural characteristics indicated for receptor sensitivity [45, 46]. The Enrichr tool is utilized to find therapeutic compounds for seven mutual DEGs. The information was retrieved from the DSigDB repository. The outcomes from the therapeutic drugs were obtained based on the P-value and the adjusted P-value. The analysis reports that Cryptolepine CTD 00001119 and piroxicam CTD 00006571 are the two drug components that most genes are connected with. As these suggested drugs were identified for the mutual DEGs, these drug molecules represent shared drugs for T2D, obesity, OC, and PD. The proposed drugs from the DSigDB repository for concordant DEGs are listed in Table 5.

#### 4. Discussion

The malfunction of metabolic activities is a result of metabolic disorders and growing concern all over the globe. Obesity has recently emerged as one of the risk factors for PD,

**Table 5.** Suggested drug compounds according to the similar genes of OC, T2D, Obesity, and PD samples

| Name of drugs             | P-value  | Adjusted P-value | Genes                          |
|---------------------------|----------|------------------|--------------------------------|
| Cryptolepine CTD 00001119 | 5.30E-08 | 2.98E-05         | CCNB1;TTK;BUB1                 |
| piroxicam CTD 00006571    | 3.07E-07 | 8.64E-05         | CCNB1;TTK;PLAT;BUB1;CD44       |
| ciclopirox MCF7 DOWN      | 1.25E-06 | 0.000179212      | CCNB1;TTK;BUB1                 |
| 5109870 MCF7 DOWN         | 1.30E-06 | 0.000179212      | CCNB1;TTK;BUB1                 |
| resveratrol CTD 00002483  | 1.70E-06 | 0.000179212      | CCNB1;TTK;PLAT;SULF1;BUB1;CD44 |
| Fulvestrant CTD 00002740  | 1.91E-06 | 0.000179212      | CCNB1;AHNAK;TTK;BUB1           |
| daunorubicin PC3 DOWN     | 4.34E-06 | 0.000331368      | CCNB1;TTK;PLAT;BUB1            |
| resveratrol MCF7 DOWN     | 4.71E-06 | 0.000331368      | CCNB1;TTK;BUB1                 |
| etoposide CTD 00005948    | 9.23E-06 | 0.000577336      | CCNB1;TTK;BUB1;CD44            |
| genistein CTD 00007324    | 1.66E-05 | 0.000933269      | CCNB1;TTK;PLAT;BUB1;CD44       |

and the indirect effects of PD on many systemic disorders have been hypothesized. In 2018, Simin Li et al. [17] conducted a network-based analysis on OC in response to PD and found that biological pathways in both OC and PD include chemokine receptors, epithelial-to-mesenchymal transition, and class I PI3K signaling events. Junho Kang et al. [47] revealed in 2022 that HGF, RAC2, PTPRC, and INPP5D hub genes were considered to be the possible therapeutic targets in their study of the association between PD and T2D. Betul Rahman et al. [48] (2023) explored the relationship between obesity and PD, reporting that groups of periodontal microorganisms were related to overweight. As T2D and obesity are both risk factors for PD and conversely, PD and OC are interconnected, the relationship among PD, obesity, OC, and T2D must be very complicated, as one is a confounding issue for the others. A network-based technique was utilized in this analysis to examine profiles of gene expression from four microarray datasets of T2D, obese, OC, and PD patients in order to discover molecular targets that potentially aid in the discovery of biomarkers for these disorders. It might also reveal vital information on their influence on newly developing disorders or illnesses. In system biology and health research, expression profiling utilizing high-throughput genome sequencing datasets has grown into an effective method for uncovering biomarker candidates for numerous illnesses. [49]. Here, the investigation of T2D, obesity, OC, and PD samples demonstrated that 7 DEGs are expressed similarly in all four disorders. On the basis of the P-values, 7 mutual DEGs were examined using Gene Ontology (GO) pathway analysis information to find insight into their biological significance in the diagnosis of T2D, obesity, OC, and PD. The common genes we have found from the above-mentioned diseases are significantly responsible for developing oral cancer [50]. We have found the DEGs for OC which have been included in supplementary file 1. The genes we have found from supplementary file 1 are common in T2D, Obesity, OC, and PD. From that perspective, the genes are correlated and all three of these disorders increase a person's risk of developing OC.

Gene Ontology (GO) offers a platform for understanding how genes and their associated pathways are regulated based on a common paradigm. Gradually, evolutions did so by acquiring a biological understanding of gene functions and their regulatory mechanisms across several ontological domains [51]. The Enrichr used the GO repository as an annotation channel in three different kinds of GO analysis: [52] biological activity, cytosolic, or molecular functionality. Negative modulation of cell migration (3 genes) and negative regulation of the intrinsic apoptotic signaling pathway in reaction to DNA degradation (2 genes) are among the top GO terms for biological functions. Negative Regulation of Cell Motility reduces, prevents, or diminishes the frequency, intensity, or relevance of cell motility [53]. In the molecular function investigation, the top two GO pathways are keratin filament binding (one gene) and hyaluronic acid binding activity (one gene). In epithelial cells, keratin-binding protein controls the AJC (apical junctional complex) and lateral domains [54]. The hyaluronic acid binding protein shows immunological cross-reactivity with

living cells, is utilized to detect the human gene mutation, and is situated on the human chromosome [55]. The leading GO terms for the cellular component are collagen-containing extracellular matrix (five genes) and focal adhesion (three genes). The extracellular matrix facilitates cell adhesion and communication with neighboring cells and it is essential for cell growth, migration, and other cellular activities. Focal adhesions are connections formed by the cell membrane and the actin cytoskeleton [56].

The most precise approach for depicting an organism's reactions to structural dynamics is pathway analysis. The KEGG pathway of 7 similar DEGs is discovered to uncover a common route for T2D, obesity, and Parkinson's disease. Viral protein association with the Cell cycle, Progesterone-mediated oocyte maturation, Oocyte meiosis, p53 signaling pathway, Complement and coagulation cascades, ECM-receptor interaction, Prostate cancer, Hematopoietic cell lineage, the FoxO signaling pathway are the top KEGG pathways and Apelin signaling pathway. Chemokine signaling is important for coordinated cell migration in health and disorder in order to precisely control the spatial and temporal placement of cells [57]. Meanwhile, WikiPathways data shows that the gene pathways involved in Senescence and Autophagy in Cancer WP615, Allograft Rejection WP2328, and Chemokine signaling pathway WP3929 are the most heavily interconnected.

PPI network analysis is the most important portion of the research, since HG finding, module analysis, and drug identification rely heavily on it. PPI analysis was also performed on the CCNB1, BUB1, TTK, PLAT, SULF1, CD44, and AHNAK genes since these are shared DEGs. The PPIs network identified the CCNB1, BUB1, TTK, PLAT, and AHNAK genes as hub genes due to the significant interaction rate or degree value shown by these genes. CD44 is a potential gene for the establishment of obesity and T2D and may be a key modulator of systemic inflammation related to T2D and obesity [58]. To emphasize the vital portions of the PPIs network, module analyses of the top leading genes were conducted. The reason for emphasizing a highly focused location is to get a more useful therapeutic drug compound prediction.

With the shared DEGs, TF-gene interactions were found. Additionally, TF genes perform the function of regulators in accordance with the genetic expressions that might contribute to the formation of cancer. According to the network, AHNAK interacts significantly with other TF genes. Within the scope of the TF-GI network, the degree value of AHNAK is 48. In the regulatory network, BUB1 and PLAT interact extensively. The degree value of BUB1 is 18, whereas the degree value of PLAT is 22. AHNAK serves an essential role in human body fat storage by stabilizing adipose tissue growth [59].

Regulatory biomolecules perform as candidate biomarkers in a variety of complicated disorders. Keeping this information in mind, the TF-mC network depicts the interactions between miRNAs and TF genes examined with respect to the regulation of shared DEGs. The analysis identified a total of 115 microRNAs and 57 TF genes. Among the TFs, the degree value of CD44 is 37 which has the most interactions. TF genes are regulators of GE, and the regulation is accomplished by interacting with target proteins, whereas miRNAs are capable of regulating gene expression by mRNA degradation [59].

According to the DSigDB database, therapeutic compounds were proposed from 7 shared DEGs. Among all potential drugs, the present analysis focuses on the 10 most important drugs. Cryptolepine CTD 00001119, piroxicam CTD 00006571, ciclopirox MCF7 DOWN, 5109870 MCF7 DOWN, resveratrol CTD 00002483, Fulvestrant CTD 00002740, daunorubicin PC3 DOWN, resveratrol MCF7 DOWN, resveratrol MCF7 DOWN, and resveratrol MCF7 DOWN are the leading drug compounds for T2D, obesity, and PD. The present investigation utilizes a variety of Bioinformatics techniques in GSE13601, GSE29221, and GSE16134, which shows OC infection, T2D infection, and PD infection in human cells, and GSE15773, which compares samples of infected and normal obesity tissue from individuals. This work eventually integrates T2D, OC, PD, and obesity treatment. These drug molecules may be examined for additional verification by chemical testing. In near future, if a larger number of samples are available, the present research would be more impactful in the context of metabolic disorders.

## 5. Conclusions

In the context of large-scale microarray data, no additional research has been conducted so far on PD, OC, T2D, and obesity. We have performed DEGs analysis among four datasets to find similar genes and determine the infection responses affected by T2D, obesity, OC, and PD. According to the findings of the bioinformatics analysis, metabolic disorders are associated with OC. Hence we are confronted with 7 common genes that are shared among these datasets. The PPI network was then produced using the concordant 7 genes, and the five most vital leading genes were discovered. From the hub genes, some drug compounds and drug-target interactions are proposed using the DSigDB database. Analysis conducted among T2D, OC, obesity, and PD provides a way for detecting infections associated with diverse illnesses. Thus, it is possible to reduce OC patients' risk of being affected by metabolic disorders. In conclusion, the outcomes of our study suggest a new path for the growth and restoration of these diseases.

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