**Results & Discussion:**

**Screening of DEGs & DEMs:**

A total 404 male patient samples ( 352 primary tumor samples & 52 solid tissue normal) were obtained from [TCGA](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE36895) & total 206 female samples (186 primary tumor & 20 solid tissue normal) were obtained from TCGA as well. After doing integrated analysis from genes of male &genes in female in the RNAseq. data, a total of 606 DEGs from male & 247 DEGS from female where obtained (using |log10 FC ≥1 and adjusted P value <0.05), including 27 upregulated genes and 579 downregulated genes in male & 12 upregulated genes in female & 235 downregulated genes in female in ccRCC samples compared to normal samples as shown in Figure1(volcano plot). Volcano plots were generated to identify the correlation between DEGs, also we get 25 DEMs from male & 29 DEMs from female.

**GO term enrichment analysis of DEGs:**

**DAVID** website was used to perform GO enrichment analysis to DEGS of male & female (the analysis include: BP, CC & MF group).

**For male DEGS**: the male DEGs were mainly enriched in multicellular organismal process, single-multicellular organism process, regulation of biological quality, single-organism transport, response to stimulus, single-organism transport, single-organism localization, single-organism process, establishment of localization, multicellular organism development, single-organism cellular process. single-organism developmental process, biological regulation. Down regulated DEGs are mainly enriched in plasma membrane, integral to membrane, endoplasmic reticulum & cytoskeleton, cell fraction, extracellular region & cytosol while up regulated genes were mainly enriched in plasma membrane, nucleus, Golgi membrane, cytosol, extracellular region & extracellular space. No significant differences were remarked between up & down regulated DEGs in their enrichment at MF, as both of them were mainly enriched in protease binding, nicotinic acid receptor activity, L-glutamate transmembrane transporter activity, protein binding, actin binding, voltage-gated potassium channel activity, signal transducer activity & calcium ion binding & voltage gated K channel activity.

**For female DEGs:** the female down regulated DEGs were mainly enriched in fatty acid metabolic process, microtubule-based process, carbohydrate transport, aromatic amino acid family metabolic process, intracellular signaling cascade, ectoderm development, M phase of mitotic cell cycle, ion transport, response to hypoxia, immune system development, cell adhesion, transmembrane transport, ------------

**Pathway enrichment analysis:**

The screened DEGs analyzed using the KEGG pathway- for the full interaction network - (Considering only P values less than 0.05). Male DEGs were enriched in Neuroactive ligand-receptor interaction, Retinol metabolism, complement & coagulation cascade, glycolysis & gluconeogenesis, Drug metabolism - cytochrome P450, Wnt signaling pathway, cell adhesion molecule, chemical carcinogenesis & Calcium signaling pathway. While in female the most enriched pathways were Collecting duct acid secretion, Retinol metabolism, Drug metabolism - cytochrome P450, epithelial cell signaling, receptor interaction& chemical carcinogenesis. From the results obtained it can be deduced that the most important pathways in male were Wnt signaling pathway, complement & coagulation cascade & metabolism of xenobiotics. Whereas the most important pathways in female were Collecting duct acid secretion & Retinol metabolism.

By performing enrichment analysis of the resulted pathways using Cytoscape to figure out the correlations between genes & the corresponding pathways, it was identified that ADH1C, VTN, PROC, CYP2B6 & ADH4 genes were involved in the most important pathways in male & ADH1C, ACPP, KCN13, SFRP1, SLC13A1genes were involved in more than one pathway in female.

**Network construction of DEGs:** The Key genes of DEGs (either male or female) & gene interactions can be identified by using STRING online database, then the results are filtered using Cytoscape software (Figure2).

**Enrichment analysis of DEGs & DEMs Using TFmir2:**  Enrichment analysis for male & female DEMs against DEGs of each one was performed to combine all TF, miRNA & gene co- regulatory interaction. We get 229 nodes & 862 edges from the full interaction network & for Disease interaction network we get 74 nodes & 150 edges from male DEGs &DEMs analysis, after using ORA analysis (of the disease interaction network) we get KEGG pathways in which male (DEGs & DEMs) are enriched in, downloading the data using **DAVID** website & visualizing it in **R** Studio we get the most important KEGG pathways ( with P value <0.05) , these pathways are : :Cell adhesion molecules (CAMs), Hippo signaling pathway, Proteoglycans in cancer, Complement and coagulation cascades, :Pathways in cancer, Melanogenesis, Basal cell carcinoma, Hepatitis C, Wnt signaling pathway, Signaling pathways regulating pluripotency of stem cells,(as shown in table 1).. By repeating the same steps for female we get--- nodes & ---edges

AS well, we identify hub genes in male & hub genes in female from the summary text of each one of them of **TFmir2,** it was found that the hub genes in male are: KNG1, ALB, AHSG, SERPINC1, IL6, APOA2, NOTUM, PROC,SERPIND1, CHRDL1, CHGB, ORM1, ITIH2, EGF, AMELX, NMUR2, CASR, PLG, GRM1, AVPR2, IGF2, HRG, PTGER1.

The hub gene for female is: TRIM63