Future Journal of Pharmaceutical Sciences

RESEARCH

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Genome-wide association study for single nucleotide polymorphism associated with mural and cumulus granulosa cells of PCOS (polycystic ovary syndrome) and non-PCOS patients

Pankaj Pant[1], Havagiray Chitme[1*], Reema Sircar[2], Ritu Prasad[3] and Hari Om Prasad[3]

Abstract

Background The genetic make-up of local granulosa cells and their function in the pathophysiology of polycystic ovary syndrome (PCOS) is crucial to a full comprehension of the disorder. The major purpose of this study was to compare the Single Nucleotide Polymorphism (SNP) of cumulus granulosa cells (CGCs) and mural granulosa cells (MGCs) between healthy individuals and women with PCOS using genome-wide association analysis (GWA). A case–control study was conducted in a total of 24 women diagnosed with PCOS and 24 healthy non-PCOS women of reproductive age aggregated into 4 samples of 6 patients each. GWA studies entail several processes, such as cell separation, cellular DNA extraction, library preparation followed by interpretation using bioinformatics databases. SNP locations were identified by reference gene also involves the use of Matrix-assisted laser desorption/ionisation-time of flight (MALDI-TOF) mass spectrometry (MS) (MALDI-TOF-MS) for the first sorting. Hybridization with the gene chip was followed by reading the SNP genotypes according to the publications in the literature. TASSEL (Trait Analysis by aSSociation, Evolution and Linkage) program and methods were used for GWA studies.

Results An aggregate of 21,039 SNP calls were obtained from our samples. Genes of autoimmune illnesses, obesity, inflammatory illnesses, nervous system diseases such as retinitis pigmentosa, autism, neural tube defects, and Alzheimer's disease; and various malignancies such as lung cancer, colorectal cancer, breast cancer were also identified in these cells. Gene ranking score reveals that granulosa cells carry key genes of neurological system and reproductive systems especially in brain and testis, respectively.

Conclusions Mural and Cumulus Granulosa cells were shown to have the PCOS directly and indirectly related genes *MMP9, PRKAA2, COMT* and *HP.* We found that the expression of *ARID4B, MUC5AC, NID2, CREBBP, GNB1, KIF2C, COL18A1*, and *HNRNPC* by these cells may contribute to PCOS.

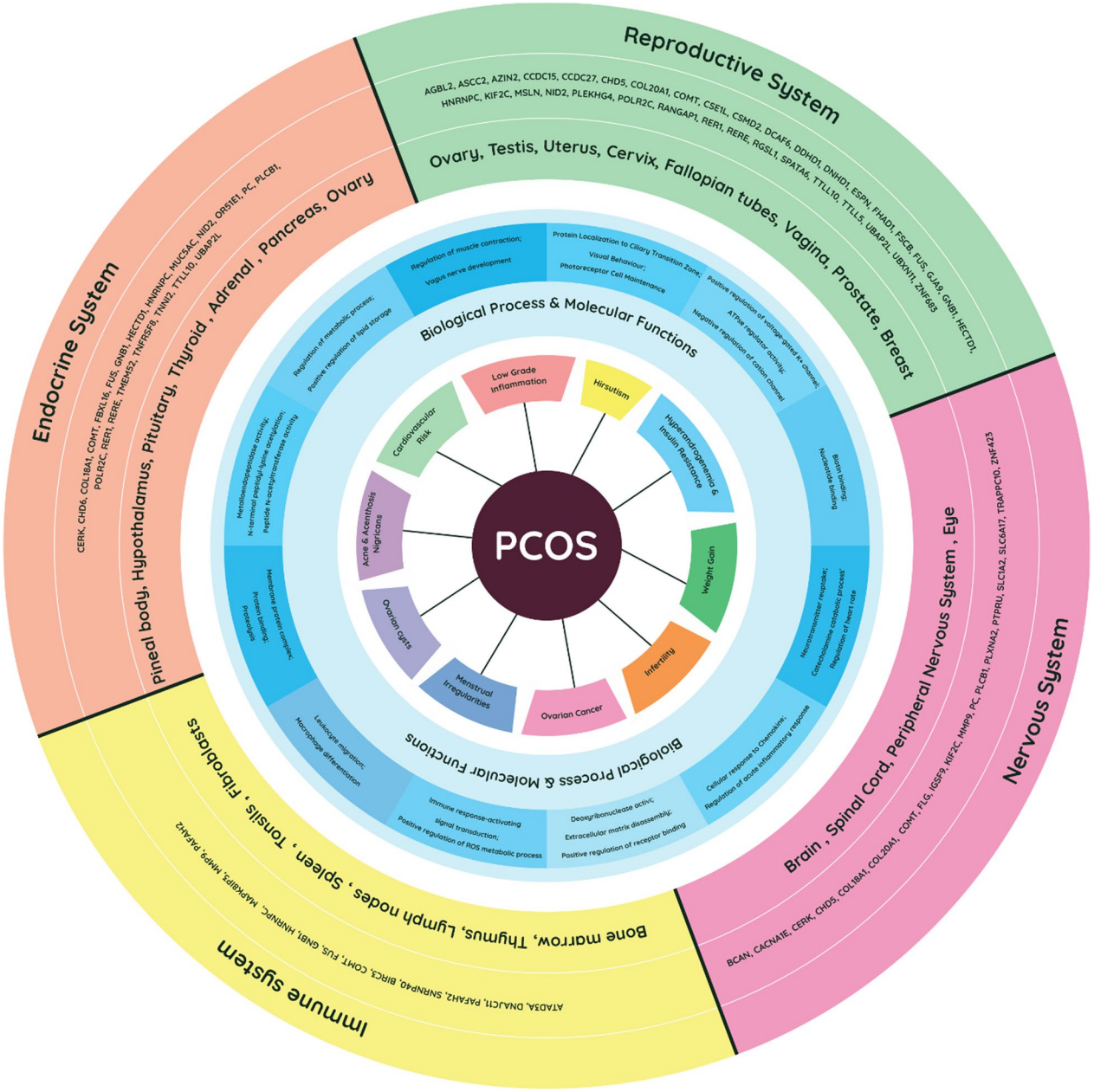
Highlights

- Investigated genetic make-up of mural and cumulus granulosa cells
- Role of these cells in pathogenesis of PCOS studied
- Local and systematic effect of these genes were correlated

*Correspondence:
Havagiray Chitme
hrchitme@gmail.com
Full list of author information is available at the end of the article

Keywords Bioinformatics, Cumulus cell, Granulosa cell, Mural cell, PCOS pathogenesis

Graphical abstract



Background

Biomedical science has changed a lot since new technologies have come out. Now, it is where many different areas of science and engineering meet to find solutions to problems that people are facing. Innovative uses of technology in healthcare can improve patient safety, clinical effectiveness, and patient monitoring, as well as help accelerate and lower the cost of developing and testing medical therapies. Some of the new and innovative technologies that are changing the healthcare system are the carbodiimide and N-hydroxy succinimide cross-linking system [1–3] thin film hydration techniques [4],

visible light photocatalysts [5], sonochemical approach [6], hydrothermal methods [7] photocatalytic compounds with band gap [8], high-performance photocatalytic process [9] and facile combustion routes [10].

Single-nucleotide polymorphisms (SNPs) are the alteration in the single base-pair sequence of the deoxyribonucleic acid (DNA) when it takes place at a high frequency of the human genome also termed as modern units of genetic variation [11]. These genetic variations are identified by cutting-edge research technique Genome-Wide Association Studies (GWASs) through comprehensive sequencing and analysis of the complete genome of thousands of SNPs at once, allowing for the identification of common SNPs that are linked to certain diseases [12]. Focusing on SNPs throughout the entire genome is an exciting new direction in the study of complex, common illnesses in which several genes contribute in identifying the genes to a person's risk, pharmacological reactions, susceptibility to contaminants and environmental factors; in prognosis and diagnosis of illness or trait [13].

Women of reproductive age (12–45 years) are disproportionately affected by polycystic ovary syndrome (PCOS), which has a varying prevalence rate across different ethnic groups (ranging from 2.2% to 26% worldwide) responsible for almost 40% of female infertility in India [14]. The clinical presentation of PCOS is more genetically variable and can have an impact on a wide range of organs including the ovary, pituitary, hypothalamus, pancreas, liver, adrenal glands, etc. Negative body image, compromised physical and mental health, and a lower quality of life are all serious concerns for women who suffer from PCOS [15]. At least 70 candidate genes have been identified as potentially contributing to the diseases of the syndrome, although the exact aetiology is still unclear [16]. PCOS symptoms include absent or irregular periods, anovulation, male-pattern hair growth, acne, acanthosis nigricans, obesity with central or distinct abdominal obesity, etc. Impaired glucose tolerance (IGT), atherogenic dyslipidaemia (AD), type 2 diabetes mellitus, hepatic steatosis, cardiovascular problems such as hypertension, and metabolic complications, as well as endometrial cancer, are all more common in these individuals [17].

During ovulation, the oocyte is accompanied by the cumulus granulosa cells (CGCs), immediately surrounding the oocyte, supporting the oocyte by sharing nutrients and a safe, caring environment. These cells emit hyaluronic acid, stabilises the produced proteins; and collectively helping the oocyte to resume meiotic division, initiating the maturation process and facilitating the formation of adult cumulus-oocyte-complexes [17]. The mural granulosa cells (MGCs) line the antrum of developing follicles surrounding the fluid-filled section of the follicle and in close proximity to the basal lamina [18]. The MGCs differentiate into luteal cells and remain in the ovary after ovulation called corpus luteum, whereas CGCs transport the egg to the oviduct. Many reports and studies have shown that the quality of oocyte production and follicular maturation are both affected by the state of these cells [19]. There are several genes present within the follicles that are differently expressed between these two divisions of granulosa cells: MGCs and CGCs [20].

Since then, CGCs and MGCs have been important in a wide range of cellular processes, such as signal transduction, making extracellular matrix, folliculogenesis, ovulation, and fertilisation. For the improvement of knowledge, many methods are used. One of these is the modern GWAS [18]. Therefore, the primary goal of this study was to look into the genome-wide association of the SNP in order to find out more about SNPs that are linked to PCOS. If these SNPs are found and proven, they could help us know and understand more about the molecular mechanism and genetic diversity of PCOS. The secondary goal of this research was to find out how different cumulus cells and mural cells are genetically and what role they play in the development of PCOS. This was carried out by looking at SNPs and gene expressions in granulosa cells with SNP arrays.

Methods

Study Participants and centre

Twenty-four women with PCOS and twenty-four control women of the same ethnicity, age range (25–37 years), and body mass index (BMI) were enrolled in the trial, which took place between January 2020 and September 2021 at Morpheus Prasad International IVF Centre and Indira IVF Fertility Centre in Dehradun. Subjects were categorised based on clinical manifestations and diagnostics, without regard to genotype. The cells' DNA was taken and combined into a pool ($n=6$), and then millions of SNPs were found. This GWA study was conducted at the TERI School of Advanced Studies, New Delhi, India.

Criterion of inclusion

The age range of the women in the research was from 25 to 37 years old. According to the Rotterdam Criteria agreement, PCOS is diagnosed when two out of three of the following conditions are present: hyperandrogenism; oligoanovulation; and polycystic ovaries on ultrasound examination (less than 12 follicles measuring 2–9 mm in diameter and/or an ovarian volume & increased ovarian volume, i.e., >10 cm^3 in a single ovary). Women without any gynaecological problems serving as a control group are those who choose to undergo the in vitro

fertilisation/Intracytoplasmic sperm injection (IVF/ICSI) process for infertility caused by male factors (husband or male partner). Women in the control group had normal ovarian morphology as determined by ultrasonography and had androgen levels within the reference range (0.4–3.5 nmol/L) throughout the follicular phase of their menstrual cycles.

Criterion of exclusion

A diagnosis of androgen-producing tumours, a lack of the enzyme 21-hydroxylase, non-classical adrenal hyperplasia, hyperprolactinemia, active thyroid disease, or Cushing syndrome would rule out participation. Medications likely to affect carbohydrate metabolism or endocrine parameters (oral contraceptives, anti-hypertensives, lipid-lowering, and anti-inflammatory drugs) for at least three months prior to entering the study were also exclusion criteria for both women with PCOS and control women. BMIs below 18 kg/m^2 or above 45 kg/m^2 were also excluded. Participants who did not sign an informed consent form were also excluded from the research.

Bioethics

All participants provided written informed consent, and the study was approved by the University Research and Ethics Committee (UREC) (DITU/UREC/2019/07/2) on July, 9th 2019. All data collected were anonymous and identity of individuals will remain confidential.

Separation of CGCs from follicular fluid

As part of IVF/ICSI, routine short or long-term gonadotropin-releasing hormone agonist injection protocols were used to stimulate the ovaries in a controlled way. After about 36 h, the follicular fluid was taken and the oocytes were taken while the patient was under a light anaesthetic. Each person's follicular fluid was taken from 3–5 follicles and put into a 15 ml polypropylene centrifuge tube with a conical bottom. The fluid was then put into Petri dishes so it could be looked at under a microscope to find cumulus oocyte complexes. After the multiple washes, both the CGCs and the pipette tips dropped off the dish. The CGCs were then mixed together and washed with 1X Phosphate Buffer Saline. Six patients' CGCs were pooled together for further study [21].

Isolation of MGCs

The rest of the follicular fluid from the previous step was put back into the tubes of the centrifuge, which contained the granulosa cells. The tubes were then spun at 800 × g for 10 min at room temperature. The supernatant was discarded, and the remaining cells at the bottom of the centrifuged tube were re-suspended with a small amount of phosphate buffer saline. This suspension was then carefully layered (to avoid mixing at this stage) over the same amount of 50% (v/v) PercollTM and centrifuged at 500 × g for 20 min at room temperature [22].The interphase layer, which is made up of MGCs because of their density, was carefully separated with a micropipette and then washed several times with 1X Phosphate Buffer Saline. Once the MGCs from six patients were collected, they were mixed together and stored at −80 °C until they could be analysed further.

DNA isolation

The genomic DNA was taken from the CGCs and MGCs according to the kit-manufacturer protocol (QIAamp DNA Mini Kit). The purity of DNA was measured by UV 260 OD/280 OD. The amount of DNA was adequate for research, and its concentration was between 20 and 50 ng/L [23].

Library preparation

Following DNA isolation, Polymerase Chain Reaction (PCR) amplification was performed using primers and a methodology similar to that published by Chen, L., Hu, M., et al. (2017), the primer sequences presented in Table 1 [18, 24].

SNP analysis

The first separation was carried out via matrix-assisted laser desorption/ionisation time of flight mass spectrometry (MALDI-TOF-MS). GCF 000001405.40 GRCh38.p14 (latest), i.e. Genome Reference Consortium Human Build 38 patch release 14 (GRCh38.p14), was used for hybridization on the HiSeq 2500 Illumina Platform with the tools; Trait Analysis by aSSociation, Evolution and Linkage (TASSEL) v5.0 pipeline, Bowtie2, FastQC, and the selection of SNPs site. Whereas Variant Call Format (VCF) and haplotype map (HapMap) files include the downloaded SNP data. To visually assess all detected variants, we utilised the Integrative Genome Viewer (IGV) (https://igv.org/app) (v2.8.9). As part of the process of identifying and prioritising disease-causing genetic variants using high-throughput sequencing data, the discovered SNPs were functionally annotated using the freely

Table 1 PCR primers

Primer name sequence	
1	1-F AGTCGATGATGCTAGCTGA
2	1-R CGTAGCTAGCTAGCTACG
3	2-F CTAGCTAGATAGCTAGCTACG
4	2-R CGATGCATATTAGCTACGATGC

F = forward; R = reverse

available web-based wANNOVAR software (http://wannovar.wglab.org/).

Interpretation method

SNPs reports from the dbSNP database (https://www.ncbi.nlm.nih.gov/snp/) were used to find and describe SNPs found in VCF files. The MalaCards database (https://www.malacards.org/) and GeneAnalytics (http://geneanalytics.genecards.org) were used as the basis for the network-based prediction method to look at the genes' expression, distribution, gene ontology, and illness correlations.

Results

Correlation of datasets

There were a total of 21,123 variations analysed by wANNOVAR, and the programme summed up the data as follows: Human Genome version 38 (hg-38) expressed 134 exomes and 21,123 genomes genes, whereas hg-19 expressed 68 exomes and 21,123 genomes genes. Only exomes are carefully chosen for the study because this part covers the majority of known disease-related variations, although it is comparatively smaller proportion of the whole genome but very significant for exploring genetic association.

Variation in genetic expression of cells

The cells of the mural and cumulus granulosa were isolated from normal and PCOS patients, respectively. When comparing cells from PCOS patients and healthy controls, the SNP investigations found no differences.

Expression of genes by systems of the body:

Analysis of results using GeneAnalytics software (https://geneanalytics.genecards.org/) in which GRCh Reference 19 and 38 were the reference genome assemblies utilised for this investigation. Taking into account that hg19 is a single representation of several genomes, we employed both hg19 and hg 38. In contrast, hg38 refers to non-standard sequences. As can be seen in Fig. 1, the majority of highly matched genes are those that are expressed in the neurological system (ranked score 2.13 and 4.09, in hg-19 & hg-38, respectively) and the reproductive system (ranked score 2.44 and 3.98, in hg-19 & hg-38, respectively). This reveals that granulosa cells carry key genes of neurological and reproductive systems.

Expression of genes in tissues

Analysis of genes using GeneAnalytics software (https://geneanalytics.genecards.org/) as shown in Fig. 2 the most common genes (*CERK, MMP9, PPP1R16B, PLCB1,*

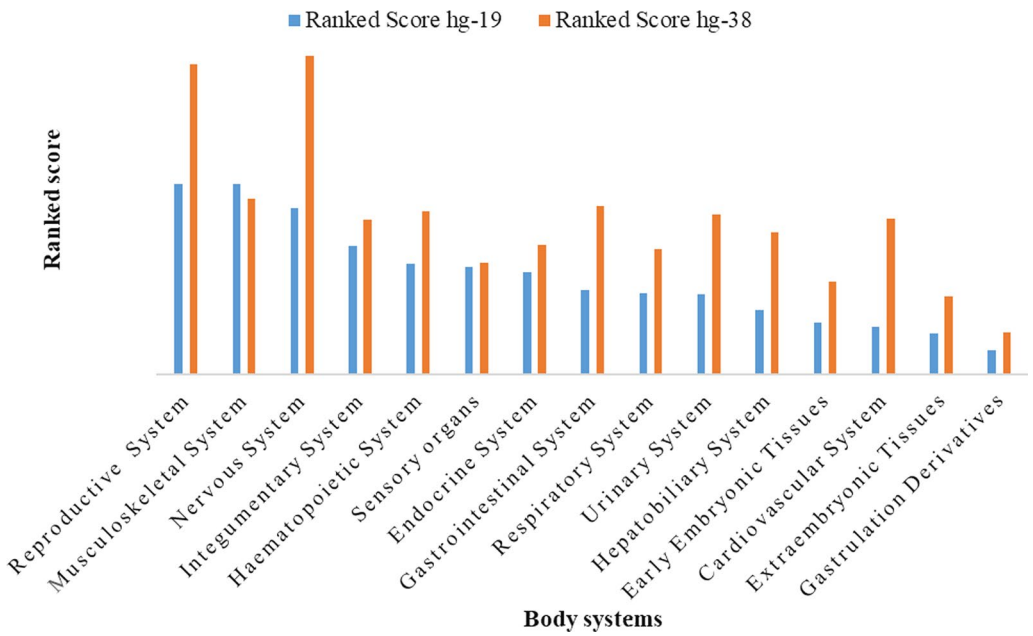


Fig. 1 Genes of Mural and Cumulus Granulosa Cells by Systems of the Body. Genes from Mural and Cumulus Cells are distributed across the many body systems in accordance with their GeneAnalytics rank score. The horizontal axis shows the various bodily systems, and the vertical bar represents the detected genes' ranking within each system; the colours reflect the hg-19 and hg-38 systems

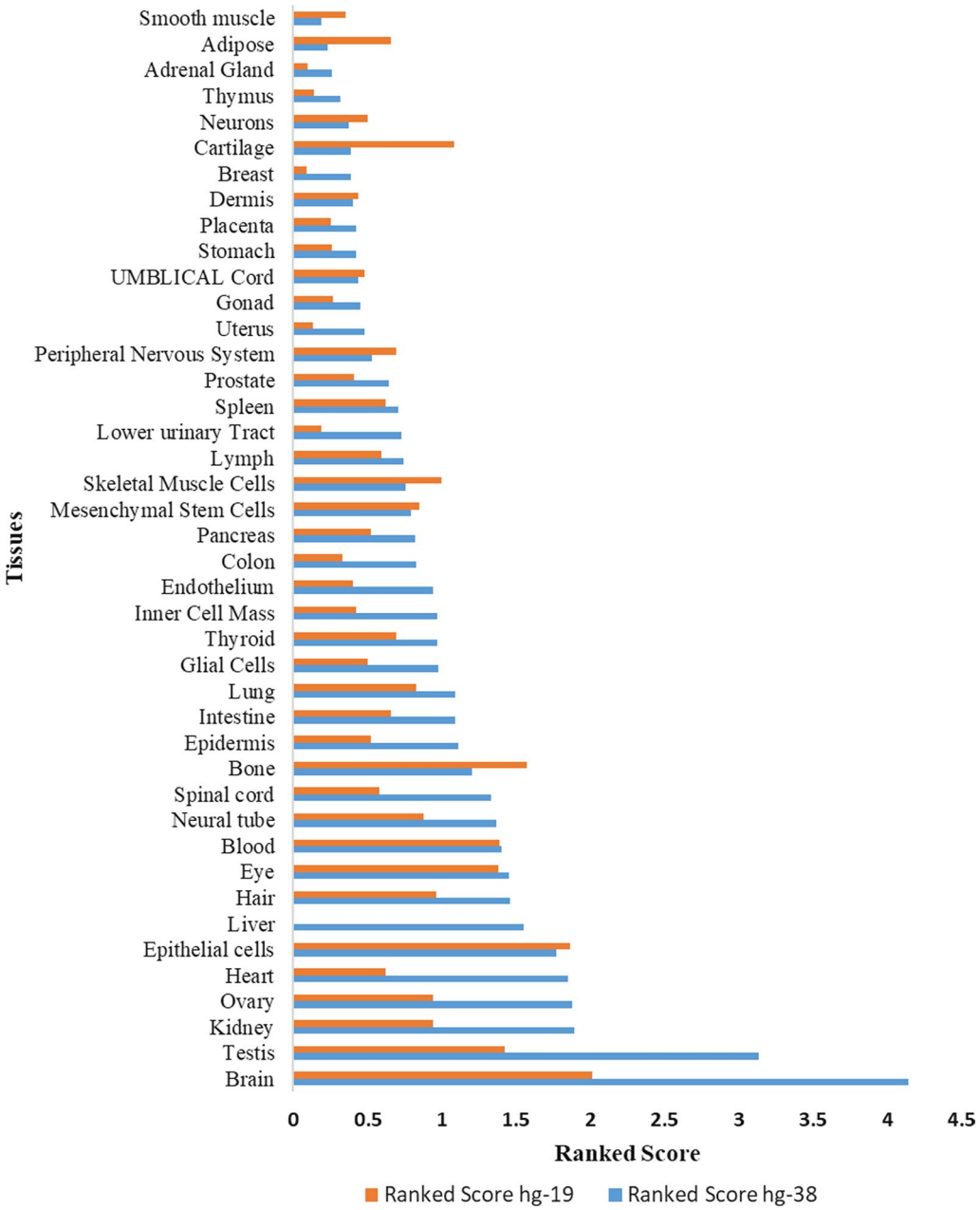


Fig. 2 Distribution of Genes of Mural and Cumulus Granulosa Cells in Tissues as per the Ranked Score. GeneAnalytics-described tissue distribution patterns for genes in murine and cumulus cells, ordered by expression level. Colour represents the hg-19 and hg-38 systems, and the horizontal bar represents the ordered score of the identified genes according to the bodily tissues along the horizontal axis

PANK2, PIEZO2, THOC1, COG4, POLR2C, NPIPB3, PPFIBP2, NAV2, NPAT, GRIK4, HNRNPC, RABGGTA, FBXL16, METRN, CCDC78, TPSAB1, DNASE1, C18orf63, etc.) identified to be expressed mainly in brain, and testis *PRKAA2, DCAF6*, and *NPAT* are all recognised to have important roles in the regulation of the coenzyme A (CoA) biosynthetic pathway. It is also noted that epithelial cells, heart, ovary and kidney (*ACAP3, GNB1, RER1, MEGF6, ARHGEF10L, PAFAH2, SNRNP40, ZC3H12A SPATA6, SLC1A7 RASSF7, TNNI2, PPFIBP2, NAV2, GRIK4, HNRNPC, CERK, SOGA1, RABGGTA)* respectively these genes are actively involved in neuromuscular junction development, immune response-activating signal transduction, vagus nerve development, negative regulation of cation channel

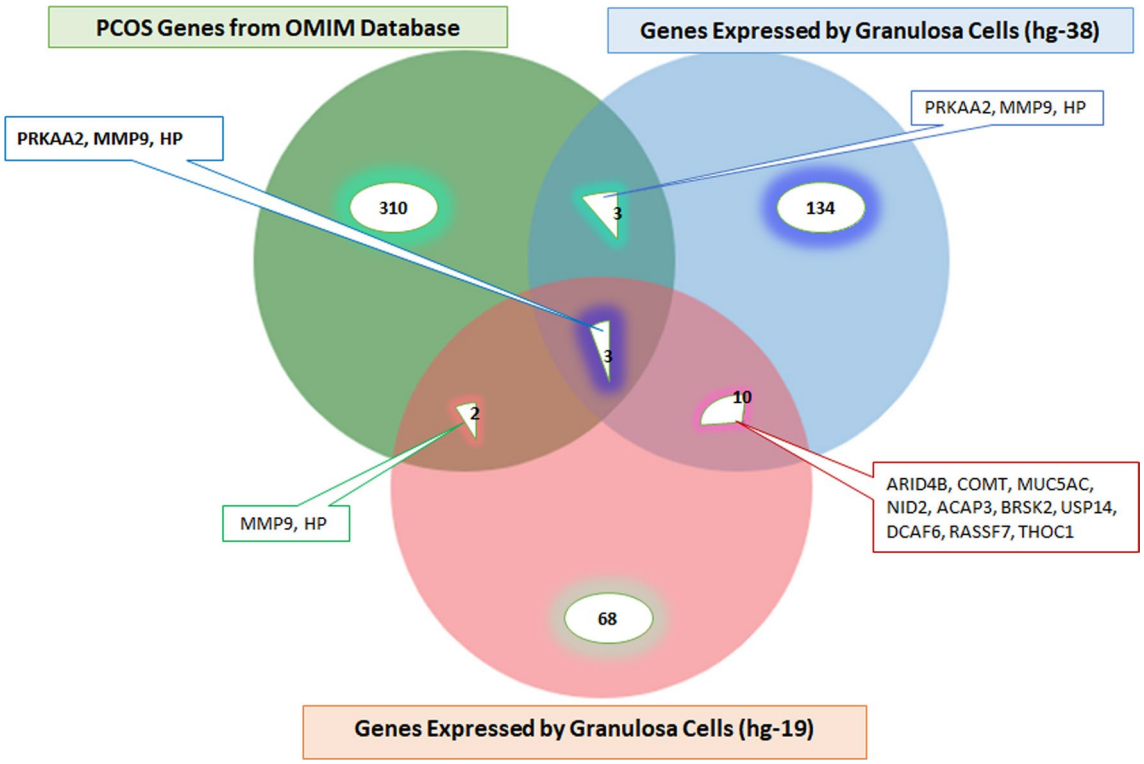


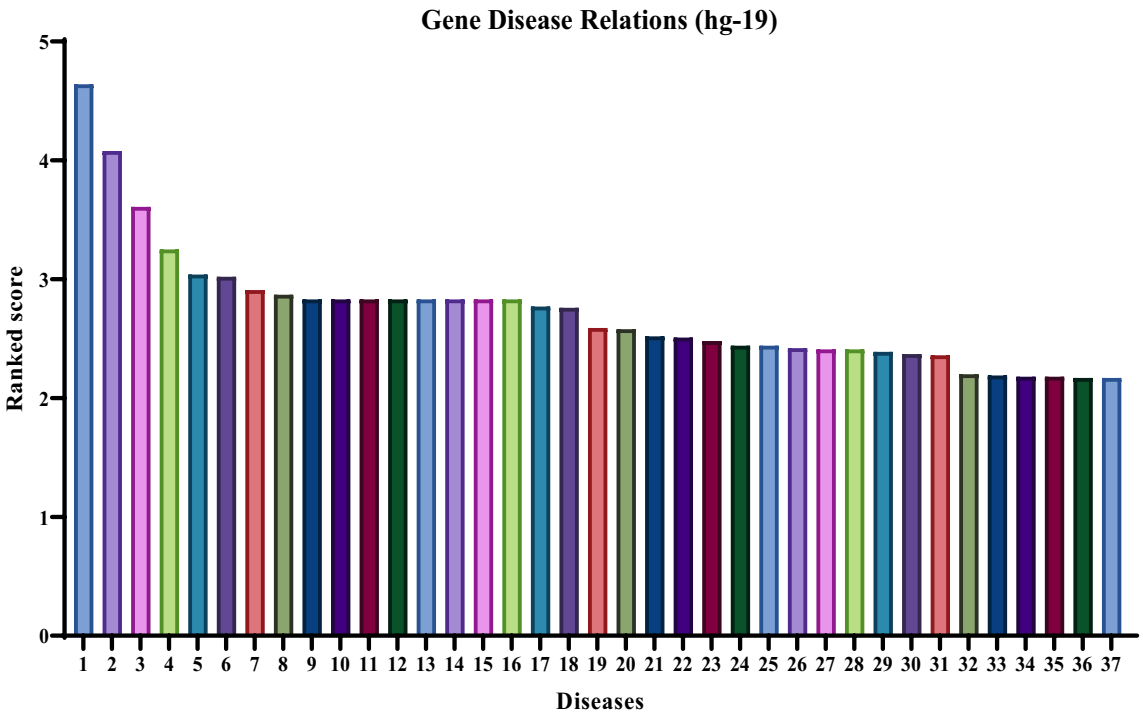
Fig. 3 Venn Diagram of Mural and Cumulus Granulosa Cells in Relations to identified PCOS Genes. The Venn diagram illustrating the connections between the genes that are expressed by the Mural and Cumulus Cells in this GWAS analysis and the PCOS Genes

activity, platelet-activating factor, acetyltransferase activity, lipid kinase activity, etc.

Venn diagram study on relationship between the genes:
Venn diagram was drawn by Bioinformatics & Evolutionary Genomics tool (https://bioinformatics.psb.ugent.be/webtools/Venn/) and data obtained from the Online Mendelian Inheritance in Man (OMIM) database (https://www.omim.org/) which shows that there are about 310 identified genes involved in PCOS (Fig. 3). When granulosa cell transcript levels were compared to reference genes, *PRKAA2, MMP9*, and *HP* were discovered to be responsible for polycystic ovary syndrome. However, in granulosa cells, both the hg19 and hg38 references share sequences for *ARID4B, COMT, MUC5AC, NID2, ACAP3, BRSK2, DCAF6, RASSF7, THOC1,* and *USP14.*

Gene disease relations
The genes obtained in this study were analysed using GeneCards (https://www.genecards.org) and GeneAnalytics software (https://geneanalytics.genecards.org/) as presented in Figs. 4 and 5 show that the greatest matching score (7.92) and the genes (*PANK2, CERK, CACNA1E, ESPN,* and *GNB*) related with Retinitis Pigmentosa. However, genes (*SHANK2, GNB, RER1,* and *TMEM52*) have been linked to fundus dystrophy, chromosome 1p36 deletion syndrome, and Autism. Retinitis pigmentosa, lung cancer, colorectal cancer, autism, cone-rod dystrophy, breast cancer, neural tube defects, Alzheimer's disease, polycystic kidney disease, follicular lymphoma, and chronic interstitial cystitis are among the top ten diseases (Figs. 5 and 6) linked to the genes found in granulosa cells.

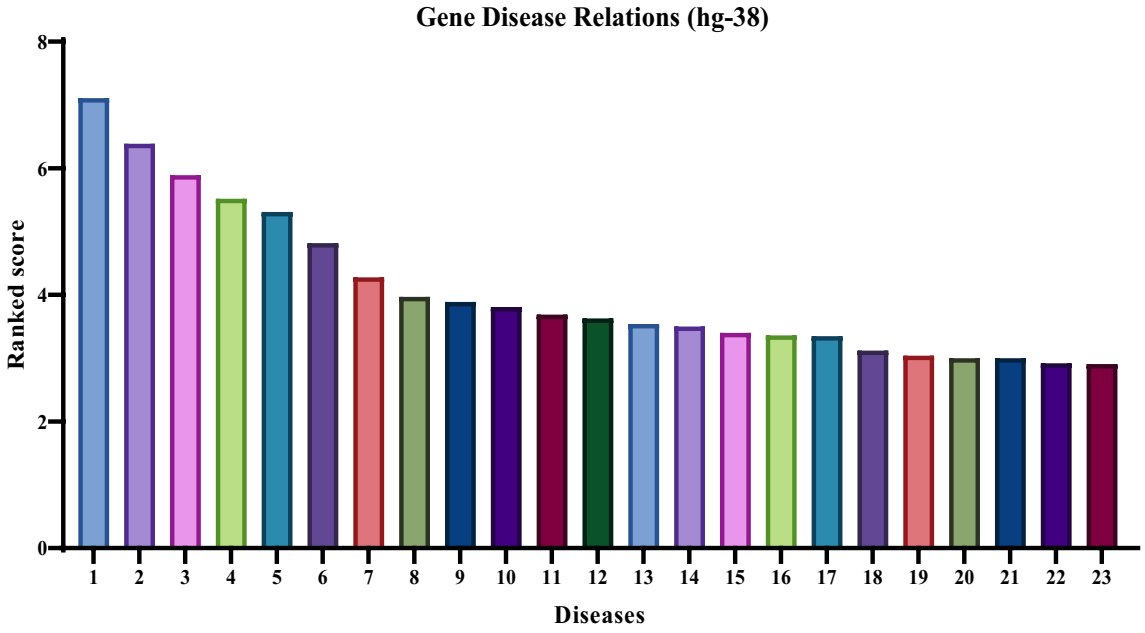
We classified illnesses into classes based on their causes and the gene families at the work (Table 2). Autoimmune illnesses have been linked to mutations in several genes, including *AAR2, ATAD3A, BCAN, BRSK2, CACNA1E, CERK, COG4, COMT, RERE, RNF207, SHANK, TTLL10, ZNF423, and many more. DNASE1, MUC5AC, MMP9, NOS1AP, TPSAB1, HP, NID2, TPSAB1, ZC3H12A* are some of the genes associated with obesity and inflammatory illnesses. *CACNA1E, GNB1, PANK2, NPHP4, FUS, RERE, SHANK2, COMT, DNHD1, SLC1A2, NOS1AP, PARS2, PIEZO2, PLXNA2, CREBBP,* and *ZNF423* are all genes that play key roles in nervous system diseases. *ARID4B, AZIN2, CDC20, CSE1L, ERMAP, FCAMR, GJB5, KIF2C, LAMB3, MAPK8IP3, MMP9, MRPL39, MSLN, PRDM2, TNFRSF8, TTLL5,* and many more have been linked to various malignancies.



1. Retinitis Pigmentosa	2. Distal Arthrogryposis
3. Cone-Rod Dystrophy 2	4.Hypotonia
5. Cystic Fibrosis	6. Dystonia
7. Schizophrenia	8. Systemic Lupus Erythematosus
9. Hypoprebetalipoproteinemia	10. Marden-Walker Syndrome
11. Nephrotic Syndrome, Type 18	12. Metaphyseal Anadysplasia 2
13. Galloway-Mowat Syndrome 8	14. Metaphyseal Anadysplasia
15. Anhaptoglobinemia	16. Intellectual Developmental Disorder
17. Microcephaly	18. Chromosome 1p36 Deletion Syndrome
19. Cerebral Visual Impairment	20. Mitochondrial Complex I Deficiency
21. Myopathy, Centronuclear, 4	22. Frontotemporal Dementia
23. Hypercalciuria, Absorptive, 2	24. Muscular Dystrophy
25. West Syndrome	26. Neural Tube Defects
27. Dicarboxylic Aminoaciduria	28. Developmental and Epileptic Encephalopathy
29. Early Infantile Epileptic Encephalopathy	30. Congenital Disorder of Glycosylation
31. Corneal Ulcer	32. Hypertension, Essential
33. Autism Spectrum Disorder	34. Dry Eye Syndrome
35. Body Mass Index Quantitative Trait Locus 11	36. Lacrimal Apparatus Disease
37. Diabetes Mellitus	

Fig. 4 Mural and Cumulus Granulosa Cell Gene Disease Relations (hg-19). The results of GeneAnalytics, which is connected to the MalaCards database, indicate the gene-disease association linkages of the Mural and Cumulus genes (hg-19)

According to Gene Ontology (GO) analyses using Gene Analytics based on hg19 as reference gene, the vast majority of granulosa cell-expressed genes play roles in biological processes and cellular activities. The majority of genes (44) are involved in protein binding; 13 genes have been localised to the extracellular area as a cellular component; and other genes play a role as cellular components in the collagen-containing extracellular



1. Retinitis Pigmentosa	2. Cone-Rod Dystrophy
3. Autism Spectrum Disorder	4. Fundus Dystrophy
5. Chromosome 1p36 Deletion Syndrome	6. Microcephaly
7. Schizophrenia	8. Colorectal Cancer
9. Essential Tremor	10. Alzheimer Disease
11. Breast Cancer	12. Skin Disease
13. Non-Specific Early-Onset Epileptic Encephalopathy	14. Congenital Nervous System Abnormality
15. Follicular Lymphoma	16. Long Qt Syndrome
17. Hypotonia	18. Body Mass Index Quantitative Trait Locus 11
19. Hypertrophic Cardiomyopathy	20. Ovarian Endometrioid Stromal Sarcoma
21. Polycystic Kidney Disease	22. Ovary Sarcoma
23. Chromosomal Deletion Syndrome	

Fig. 5 Mural and Cumulus Granulosa Cell Gene Disease Relations (hg-38). Results from GeneAnalytics (connected to the MalaCards system) reveal the genetic links between Mural and Cumulus-related diseases (hg-38)

matrix, a cellular anatomical entity. Cellular response to glucose deprivation is studied, and the proteins *BRSK2, SOGA1,* and *PC* are found to be involved. There are 81 genes engaged in protein binding at the molecular level, and 45 genes involved in the cytoplasm as cellular components, when GO investigations are compared to the reference hg38. It has been found that the expression of three genes, *CACNA1E, NOS1AP,* and *RNF207,* positively regulates voltage-gated potassium channel activity. According to GO analyses based on hg19, most granulosa cell-expressed genes have roles in some sort of biological function (Figs. 7 and 8). Mural and cumulus cells participate in biological processes, as shown in Fig. 9. These cells are involved in protein binding, ATP binding, and ATP hydrolysis activities.

Protein–Protein interaction

String diagrams were drawn using string.com to learn how granulosa cell-expressed genes interact with one another at the protein–protein level (Fig. 10). *CREBBP* was centric to the whole process. Other important interacting clusters include *GNB1, KIF2C, COL18A1, MMP9,* and *HNRNPC.*

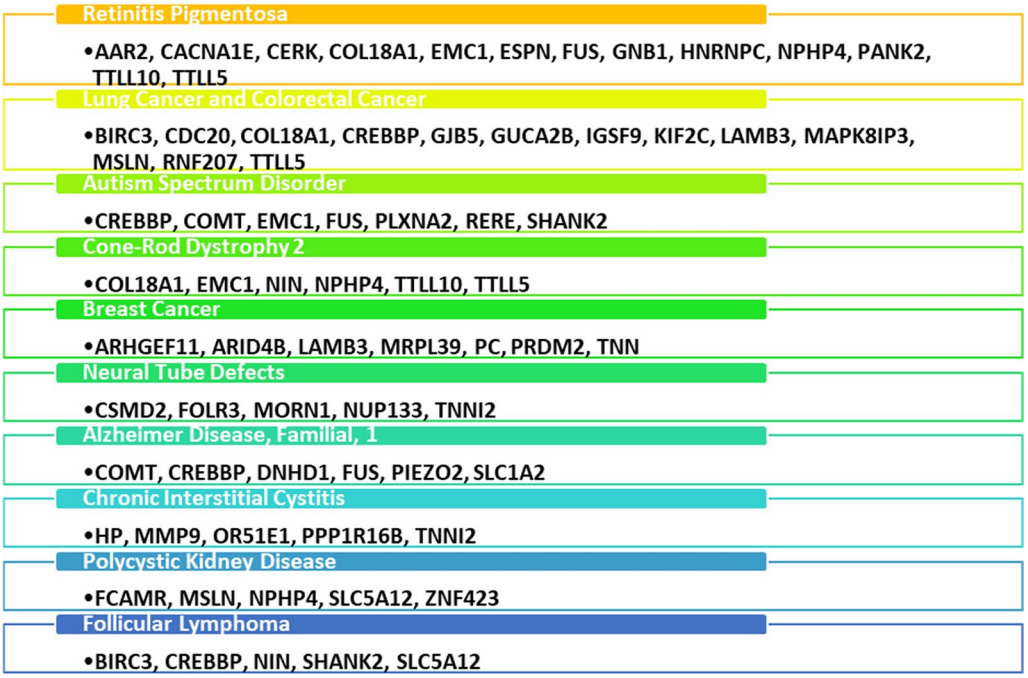


Fig. 6 Top 10 most common diseases associated with the genes of mural and cumulus granulosa cells. According to MalaCards and GeneAnalytics, these are the top 10 most prevalent diseases that are connected with the genes found in mural and cumulus granulosa cells

Discussion

The study's main and secondary goals were met by analysing gene expressions using SNP arrays to define the genomic diversity of CGCs and MGCs and their role in pathogenesis, and by doing a GWA study to learn more about pathogenesis of PCOS as mentioned in the previous study that it provides integrative genetics information [74]. Nearly a billion variants have been mapped to hg38 (GRCh38), and their different places on the genome add up to more than 1.06 billion sites supporting the claim of phenotypes and genotypes leading to potential functions [75]. After remapping the dbSNP variants from hg-38 to hg-19 (GRCh-37), on the other hand, about 981 million new variants with 1.02 billion different genomic locations were added due to higher neutral mutation rates mentioned in the literature [76]. To identify the similarities and differences between variants we used both hg19 and hg38 because hg19 is a unified representation of several genomes and hg38 is a representation of alternative sequences in line with previous study [77]. The genes expressed in the nervous system (2.13 and 4.09 in hg-19 and hg-38, respectively) and the reproductive system have the highest matching scores (2.44 and 3.98 in hg-19 and hg-38, respectively). This shows that granulosa cells have the genes needed for these systems substantiate the fact that they contribute in pathogenesis of PCOS through hyperandrogenism, obesity and insulin resistance [78].

Insulin resistance is known to be linked to PCOS through Protein Kinase AMP-Activated Catalytic Subunit Alpha 2 (*PRKAA2)*, which is also found in granulosa cells. Our study also found Matrix Metallopeptidase 9 (*MMP9*) is linked to a higher risk of cardiovascular disease, which is also a serious problem that can happen with PCOS in line with earlier study [79]. Since haptoglobin (*HP*) is responsible for anti-oxidant and anti-inflammatory properties, its levels may be lower in people with PCOS supporting the fact that it has significant pro-inflammatory role in diabetes and metabolic syndrome [80]. Through GWA studies of SNPs, the results of this study showed the presence of *PRKAA2, MMP9,* and *HP*. This shows that granulosa cells are expressing these genes, which may lead to PCOS and complications.

The results of this study show that the granulosa cells have genes that make *HP*, which binds to haemoglobin (Hb) and causes haemolysis when macrophages recognise this. It shows that the *HP* gene in granulosa cells is linked to PCOS problems due to insulin resistance mentioned in previous study [81]. The most found genes Ceramide Kinase (*CERK*), *MMP9,* Protein Phosphatase 1 Regulatory Subunit 16B (*PPP1R16B*), Phospholipase C Beta 1 *(PLCB1),* Pantothenate Kinase 2 *(PANK2),* Piezo Type Mechanosensitive Ion Channel Component 2*(PIEZO2),* THO Complex Subunit 1 *(THOC1),* Component of Oligomeric Golgi Complex 4 *(COG4),* RNA Polymerase II Subunit C *(POLR2C),* Nuclear Pore Complex Interacting

Table 2 Body system genes expressed in granulosa cells and their association with systemic diseases

Disease category	Disease	Genes	Phenotypic	Gene ontology
Genetic	Autosomal Dominant Non-Syndromic Intellectual Cerebellar Hypoplasia/atrophy, Epilepsy, and Global Developmental Delay Chromosome Deletion Syndrome Cone-rod dystrophy [2] Congenital disorder of Glycosylation Cystic fibrosis Hypertrophic Cardiomyopathy Nephrophthisis [14] Retinitis Pigmentosa West syndrome	*AAR2* [25], *ATAD3A* [26], *BCAN* [27], *BRSK2* [28], *CACNA1E* [29], *CERK* [30], *CFAP74* [31], *CHD5* [32], *COG4* [33], *COL18A1* [34], *COMT* [35], *DNASE1* [36], *EMC1* [37], *ESPN* [38], *FLG* [39], *FUS* [40], *GNB125*, *HP* [41], *MMP9* [42], *MUC5AC* [43], *NOS1AP* [44], *NUP133* [45], *PC* [46], *PIEZO2* [47], *RERE* [48], *RNF207* [49], *SHANK2* [50], *SLC1A7* [51], *TNNI2* [52], *TTLL10* [53], *ZNF423* [54]	Abnormal myelination Autism Cognitive impairment Decreased circulating glucagon level EEG Abnormality Epidermal spongiosis Gastrophageal reflux Growth delay Hyperactivity Hypermetropia Intrauterine growth retardation	Immune response-activating signal transduction Neurotransmitter reuptake Nucleotide binding +ve regulation of voltage-gated K + channel activity Regulation of heart rate by chemical signal Regulation of muscle contraction Skeletal muscle contraction Vagus nerve development Visual behaviour
Autoimmune	Alzheimer's Disease Autism Spectrum Disorder Dystonia Non-specific early-onset epileptic encephalopathy Rheumatoid arthritis Spastic paraplegia [47] Systemic lupus erythematosus West syndrome	*AAR2, ATAD3A, BRSK2, CACNA1E, CCDC78, COL18A1, COMT, CREBBP, DNASE1, DNHD1, FLG, FUS, GNB1, HP, MMP9, MUC5AC, NAV2, NIN* [55], *NOS1AP, NPHP4, NUP133, OBSCN, PANK2, PARS2* [56], *PIEZO2, PLCCB1, PLXNA2* [57], *PPP1R16B, RANGAP1, RERE, SHANK2, SLC1A2, SLC1A7, TNNT2, USP14* [58], *ZC3H12A* [59], *ZNF423*	Abnormal central motor function Abnormal muscle tone Autosomal dominant inheritance Generalised hypotonia Hip dysplasia Hyperactivity Hypotonia Short Stature	Acute inflammatory response Antioxidant activity ATPse regulator activity Basement membrane organisation Cellular response to Chemokine Immune Response-activating signal transduction Macrophage differentiation Metalloendopeptidase activity Neurotransmitter reuptake +ve regulation of lipid storage +ve regulation of voltage-gated K + channel activity Protein binding
Obesity	Body mass index, Quantitative trait locus [11] Myocardial infraction Osteoarthritis	*DNASE1, HP, MMP9, NID2, TPSAB1* [60], *ZC3H12A*	Abnormal acute inflammation Antinuclear antibody positivity Aplasia / hypoplasia involving pelvis Autoimmune antibody positivity Increased IgG3 level Increased interleukin secretions Lupus nephritis Malar rash Pericarditis Pleuritis Serositis	Leukocyte migration Macrophage differentiation Metalloendopeptidase activity -ve regulation of cation channel activity +ve regulation of lipid storage +ve regulation of ROS metabolic process +ve regulation of receptor binding Proteolysis Protein binding Regulation of acute inflammatory response

Table 2 (continued)

Disease category	Disease	Genes	Phenotypic	Gene ontology
Cancers	Breast Cancer Colorectal Cancer Corneal ulcer Lung Cancer Lymphoma Myelodysplastic syndrome Prostate Cancer Wilms Tumour [5]	*ARID4B, AZIN2* [61], *CDC20* [62], *CSE1L* [63], *ERMAP* [64], *FCAMR* [65], *GJB5* [66], *KIF2C* [67], *LAMB3* [68], *MAPK8IP3* [69], *MMP9, MRPL39, MSLN* [70], *PRDM2* [71], *TNFRSF8* [72], *TTLL5*	Decreased sensitivity to xenobiotic induced morbidity/mortality Hypermetropia Increased circulating interferon-gamma level Increased IgG3 level Increased interleukin secretions Renal cyst	ATPse regulator activity Biotin binding Catecholamine catabolic process Deoxyribonuclease activity ECM disassembly Immune Response-activating signal transduction Membrane protein complex N-terminal peptidyl-lysine acetylation Ornithine metabolic process Peptide N-acetyltransferase activity +ve regulation of voltage-gated K+ channel activity Regulation of neutrophil mediated cytotoxicity
Inflammatory	Acute dacryocystitis Asthma Blepharitis Senile ectropion	*DNASE1, MUC5AC, MMP9, NOS1AP, TPSAB1*	Abnormal acute inflammation Abnormal histamine physiology Abnormal interleukin-12 secretion Abnormality of upper limb metaphysis Decreased mast cell protease storage Hypoproteinemia Increased circulating interferon-beta level Increased circulating interferon-gamma level Increased IgG3 level Increased interleukin secretions Lupus Nephritis Microscopic Haematuria	Cellular response to ROS ECM disassembly Leukocyte migration Macrophage differentiation Metalloendopeptidase activity -ve regulation of cation channel activity -ve regulation of epithelial cell differentiation involved in kidney development Neutrophil activation involved in immune response NO synthase activity Phosphatidylinositol-mediated signalling +ve regulation of voltage-gated K+ channel activity +ve regulation receptor binding Proteolysis Regulation of neuroinflammatory response Regulation of neutrophil mediated cytotoxicity
Nervous System Disease	Alzheimer's Disease Amyotrophic Lateral Sclerosis Essential tremor Glaucoma Intracranial berry aneurysm Microcephaly Myopathy Schizophrenia Spastic paraplegia [47]	*CACNA1E, GNB1, PANK2, NPHP4* [73], *FUS, RERE, SHANK2, COMT, DNHD1, SLC1A2, NOS1AP, PARS2, PIEZO2, PLXNA2, CREBBP, ZNF423*	Cerebral degeneration Mental deterioration Neuronal loss in the cerebral cortex Nystagmus Pallidal degeneration Postnatal growth retardation Scoliosis Social and Occupational deterioration Subcortical cerebral atrophy	Catechol O-methyltransferase L-dopa O-methyltransferasec -ve regulation of cation channel activity Neurotransmitter reuptake Peptide N-acetyltransferase activity Regulation of heart rate Skeletal muscle contraction Synaptic receptor adaptor activity Vagus nerve development

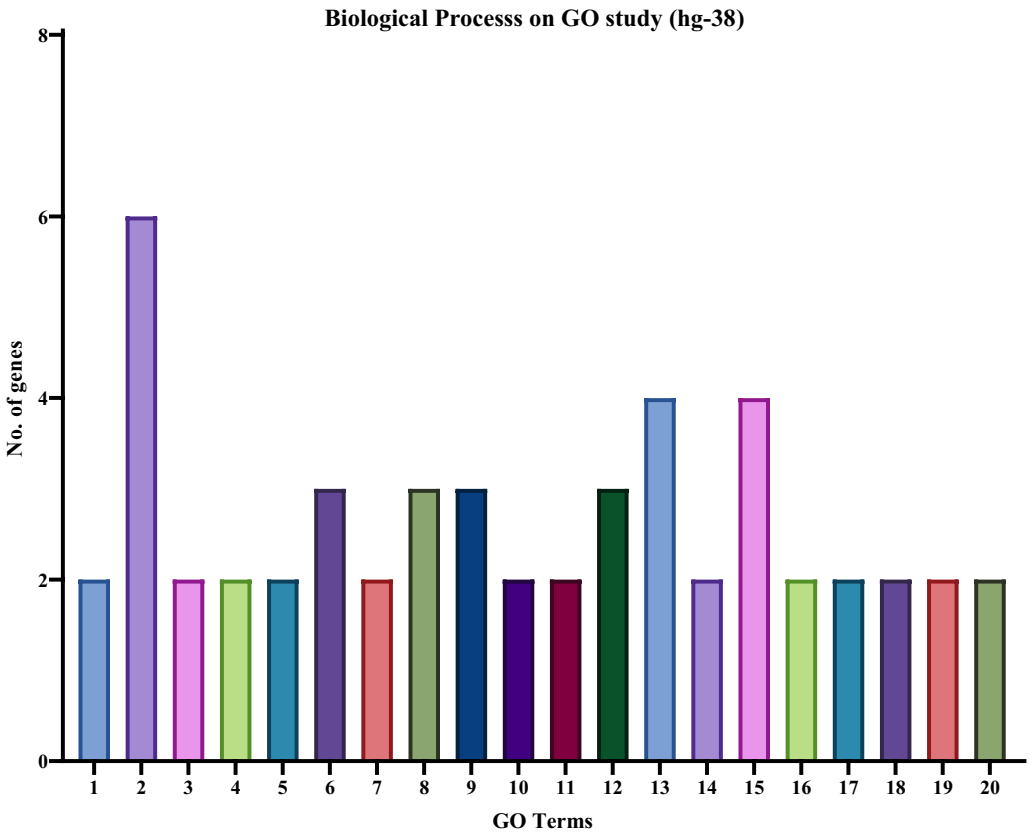


Fig. 7 Biological Process on GO Study (hg-38). The Gene Ontology enrichment analysis of the Mural and Cumulus Granulosa Cells. The number of genes that are more prevalent in each biological process is represented along the vertical axis of the bar (hg-38)

Protein Family Member B3 *(NPIPB3),* PPFIA Binding Protein 2 *(PPFIBP2),* Neuron Navigator 2 *(NAV2),* Nuclear Protein, Coactivator of Histone Transcription *(NPAT),* Glutamate Ionotropic Receptor Kainate Type Subunit 4 *(GRIK4),* Heterogeneous Nuclear Ribonucleoprotein C (*HNRNPC*), Rab Geranylgeranyltransferase Subunit Alpha *(RABGGTA),* F-Box And Leucine Rich Repeat Protein 16 (*FBXL16*), Meteorin, Glial Cell Differentiation Regulator (*METRN*), Coiled-Coil Domain Containing 78 (*CCDC78*), Tryptase Alpha/Beta 1 (*TPSAB1*), Deoxyribonuclease 1 (*DNASE1*), etc.) are mostly expressed in the brain, ovary, and testis. These supports the fact that there is a variation in expression of individual genes in organs [82]. Genes shared by epithelial cells, hearts, ovaries, and kidneys ArfGAP With Coiled-Coil, Ankyrin Repeat and PH Domains 3 (*ACAP3*), G Protein Subunit Beta 1 (*GNB1*), Retention In Endoplasmic Reticulum Sorting Receptor 1 *(RER1),* Multiple EGF Like Domains 6 *(MEGF6),* Rho Guanine Nucleotide Exchange Factor 10 Like *(ARHGEF10L),* Platelet Activating Factor

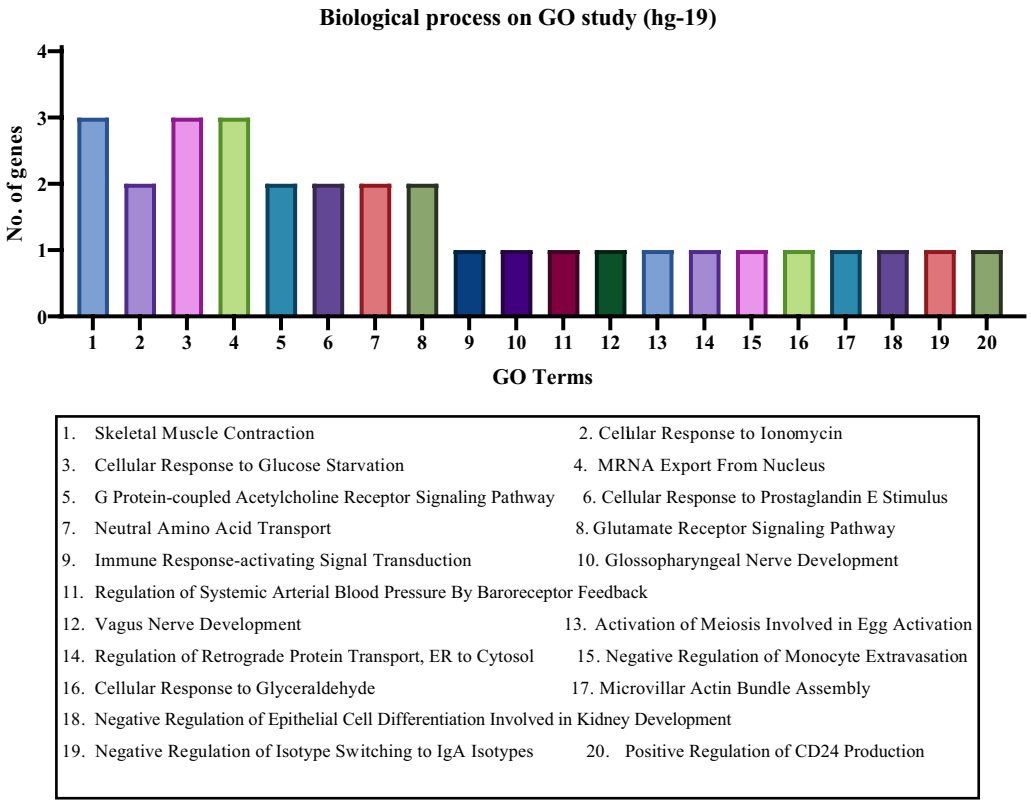


Fig. 8 Biological Process on GO Study (hg-19). The examination of the enrichment of Gene Ontology for the Mural and Cumulus Granulosa Cells. The number of genes that are more prevalent in each biological process is represented along the vertical axis of the bar (hg-19)

Acetylhydrolase 2 (*PAFAH2*), Small Nuclear Ribonucleoprotein U5 Subunit 40 (*SNRNP40*), Zinc Finger CCCH-Type Containing 12A (*ZC3H12A*), Spermatogenesis Associated (*6SPATA6*), Solute Carrier Family 1 Member 7 (*SLC1A7*), Ras Association Domain Family Member 7 (*RASSF7*), Troponin I2, Fast Skeletal Type (*TNNI2*), *PPFIBP2, NAV2, GRIK4, HNRNPC, CERK,* Suppressor Of Glucose, Autophagy Associated 1 (*SOGA1*), and *RABGGTA*) are involved in neuromuscular development, neurotransmitter reuptake, positive regulation of miRNA catabolic process, immune response-activating signal transduction, vagus nerve development, etc., as mentioned in previous study [83]. It is possible that these genes, which are found in granulosa cells, are involved in the altered physiological activities that occur with PCOS.

We found six genes that are common between hg19 and hg38 by comparing the SNP data from the two genomes. These genes include AT-Rich Interaction Domain 4B(*ARID4B*), *COMT, HP, MMP9, NID2* and Mucin 5AC, Oligomeric Mucus/Gel-Forming(*MUC5AC*). *ARID4B* of granulosa cells, which is expressed in the testis, bone marrow, bladder, and adrenal glands, among other places, and is responsible for the overexpression of testosterone from sertoli cells, may be the cause of hyperandrogenism [84].This links to the development of hyperandrogenism commonly seen in PCOS. Placental, adrenal, and ovarian Catechol-O-methyltransferase (*COMT*) expression is first implicated in symptoms of PCOS such as fibromyalgia, chronic fatigue, and mild inflammation. As per the literature and results of present study these genes were also found in granulosa cells, which indicates that a significant role may be played by the genes of granulosa cells [85]. The PCOS-related behaviours of diabetic nephropathy, coronary artery disease, and inflammatory illness were all discovered in granulosa cells, indicating that *HP* may have a role in the aetiology of PCOS similar to the fact produced in earlier study they were found strongly associated with obesity and glucose tolerance [42]. The *MMP9* gene has a role in the remodelling of tissue, reproduction, embryonic development, and the local proteolysis of the extracellular matrix, as well as in the differentiation of macrophages and the migration of leukocytes. These study outcomes validate the role of this gene in reduction in scar formation and promote neovascularization [86]. PCOS is a syndrome in which there is a disruption in the normal functions of the immune system, the development of embryos, reproduction, and the remodelling of several different tissues, organs, and

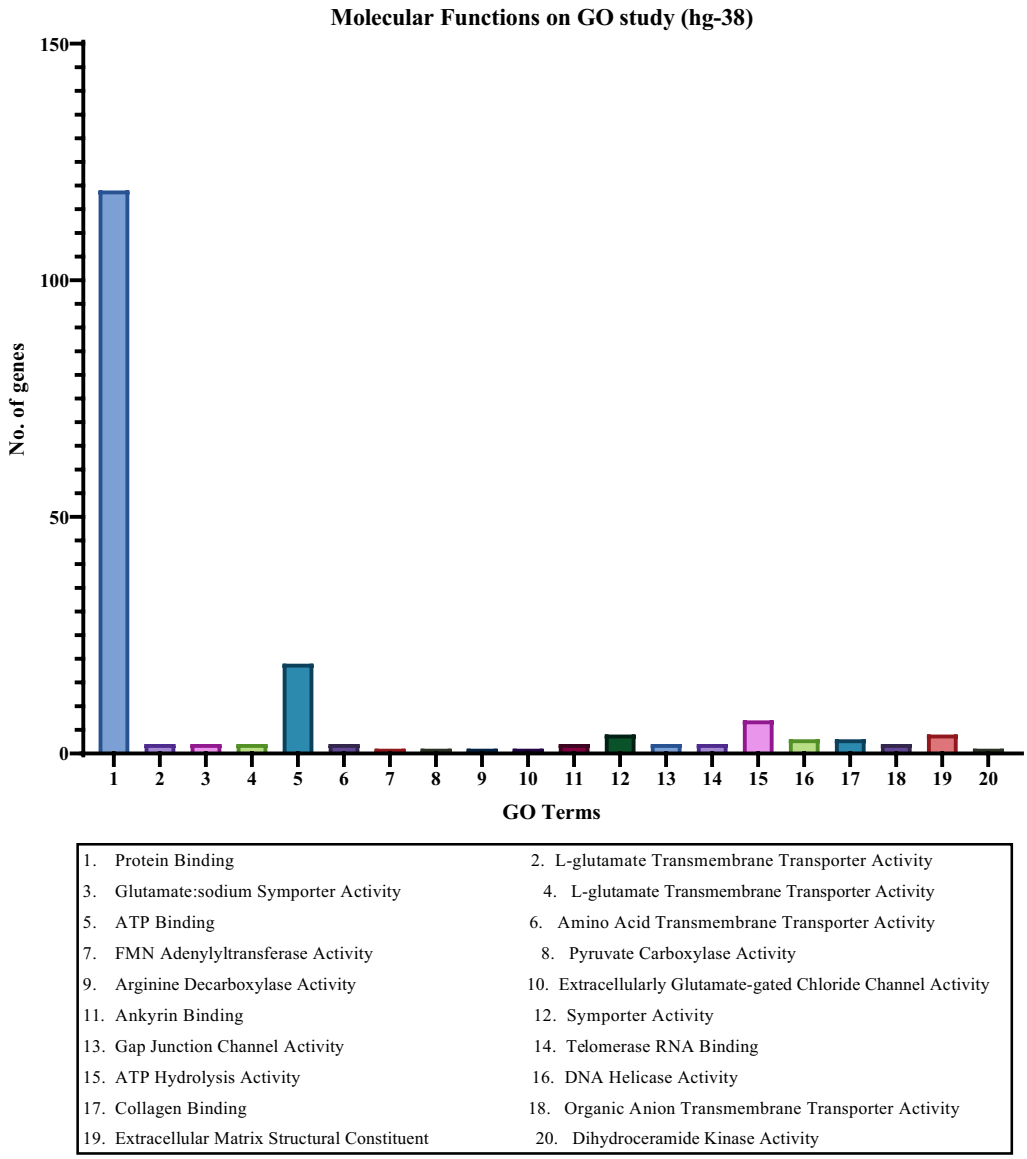


Fig. 9 Gene Ontology Study on Molecular Functions of Mural and Cumulus Granulosa Cells. The number of genes enriched in each molecular function is plotted along the vertical axis of the Molecular Functions bar (hg-38)

systems. This provides some insight into the potential function of these genes in granulosa cells. The expression of Mucin 5AC, Oligomeric Mucus/Gel-Forming (*MUC5AC*) in the stomach has been connected to biliary tract sickness, itchy-dry eye disease, Sjogren's syndrome, cystic fibrosis, and other disorders implicated with PCOS [87]. In our study *MUC5AC* expression found in CGCs and MGCs. The fact that *NID2*, which is linked to cell-adhesion, cardiovascular disease, polycystic ovarian syndrome, gastric cancer, and other diseases and conditions, is expressed in these granulosa cells is an indication that MGCs and CGCs are involved in PCOS pathogenesis through expression of this gene. Earlier study correlated other diseases and conditions, such as nasopharyngeal, esophageal, and oral carcinoma linking to *NID2* methylation [88].

The Cyclic adenosine monophosphate (cAMP) response element-binding protein (*CREBBP*) gene plays a vital role in the interactive signalling process, as shown by the findings of studies on the interactions between proteins. It might be because of its essential function in the cellular phosphorylation signalling system, which influences processes such as cell differentiation, proliferation, growth, and cyclic regulation as mentioned in previous study [89]. If these steps are changed in PCOS, it could be because the expression of genes in granulosa cells has changed.

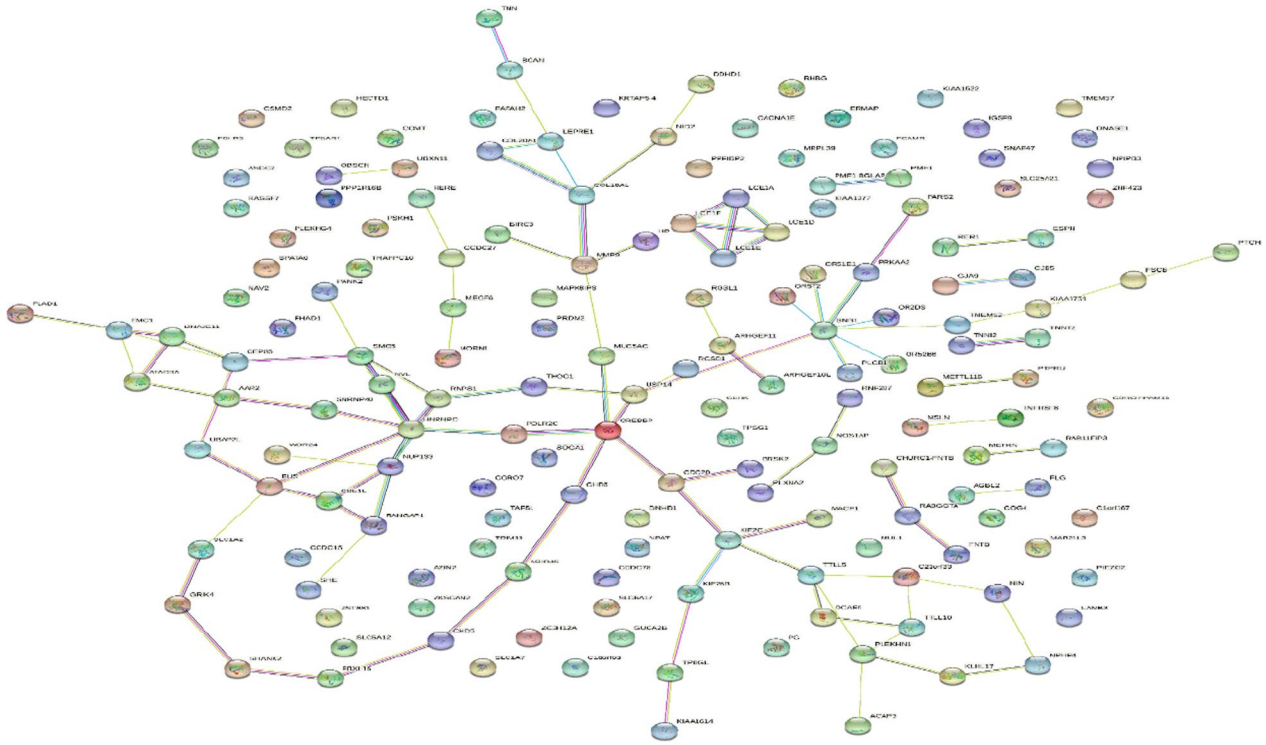


Fig. 10 String Diagram on Protein–Protein Interaction. The genes that make up the network's centre nodes (the main genes) and its edges (the genealogical connections between proteins and their pathways) are displayed

GNB1, which we found, acts as a modulator or transducer in several transmembrane signalling pathways that control cell growth and differentiation, transport activities, protein synthesis, and other processes affected by PCOS. This is in line with the outcome of previous study explaining its importance in clinical etiopathogenesis of mutation [90]. Another gene of Kinesin family member 2C (*KIF2C*) is involved in chromosomal segregation during mitosis reflected in previous study [91] was also present in granulosa cells which is one of the most common complications in PCOS. In this GWA study, we found that Collagen alpha-1(XVIII) chain (*COL18A1*), which is involved in extracellular matrix architecture, Phospholipase-C pathway, collagen chain trimerization, ERK signalling, etc., similar to hepatotoxicity induced by drugs presented by previous study [92] affects the signalling process in PCOS, showing the role of this gene in the signal transduction pathway in skeletal muscle, which causes a change in glucose uptake as mentioned in earlier study [93].

Heterogeneous nuclear ribonucleoproteins C1/C2 (*HNRNPC*) is an oncogenic gene that is found in a wide range of tissues and organs, such as bone marrow, brain, ovary, bladder, testis, thyroid, endometrium, etc. These results are supported by one of the study carried out on *hnRNP* family [94, 95]. It is involved in protein metabolism, signalling through *RhoGTPases*, and the Rho Related BTB Domain-GTPase (*RHOBTB-GTPase)* Cycle. It was also found in MGCs and CGCs. Because of this gene, the cyst might continue to recur even after surgery as reported in earlier study [96, 97].

Conclusion

We have compiled a list of the characteristics of a large number of genes that are present in MGCs and CGCs, as well as their direct and indirect connections to PCOS. Based on the results, we can propose that the MGCs and CGCs express genes *PRKAA2, MMP9, HP, ARID4B, COMT, MUC5AC, NID2, CREBBP, GNB1, KIF2C, COL18A1,* and *HNRNPC* mostly connected to the clinical symptoms of PCOS, such as insulin resistance, cardiovascular disease, low-grade inflammation, hyperandrogenism, fibromyalgia, chronic fatigue, and itchy- dry eyes. We do suggest, though, that more detailed studies be done with a larger number of patients from different hospitals, regions, and ethnic groups. This would give us more information from which to reach a conclusion on the role of CGCs and MGCs in pathogenesis of PCOS.

Abbreviations

BMI	Body mass index
CGCs	Cumulus granulosa cells
COCs	Cumulus oocyte complexes
COMT	Catechol-O-methyltransferase
DNA	Deoxyribonucleic acid
FSH	Follicle-stimulating hormone
GLM	Generalised linear models
GO	Gene Ontology
GWAS	Genome-Wide Association Study
hCG	Human chorionic gonadotropin
ICSI	Intracytoplasmic sperm injection
IVF	In vitro fertilisation
LH	Luteinizing hormone
MGCs	Mural granulosa cells
MLM	Mixed linear models
OMIM	Online Mendelian Inheritance in Man (OMIM®)
PCOS	Polycystic ovary syndrome
SNP	Single nucleotide polymorphism
VCF	Variant Call Format

Acknowledgements
We take this opportunity to thank all volunteers involved in the study. We also acknowledge thanks to Mr. Ajeet Sharma, Mr. Nitin Kataria, Mr. Achal Tiwari, Ms. Laxmi Arya and all other technical and non-technical staff of Morpheus Prasad International IVF Centre and Indira IVF Fertility Centre, Dehradun for their help and support throughout the study. We also thank Dr. Shashi Bhushan Tripathi, TERI School of Advanced Studies, Delhi and Dr. Anoop Anand Malik, TERI, Guwahati. This research was financially supported by the Bayer Pharmaceuticals, Germany and Seed Grant of DIT University, Dehradun—India.

Author contributions
All authors have equally contributed in conducting the study and prepare the manuscript. All authors read and approved the final manuscript.

Funding
This research was supported by the Bayer Pharmaceuticals (Grant No. 2019-09-2438), Germany and Seed Grant of DIT University, Dehradun—India.

Availability of data and materials
Data and materials are confidential and available with first author.

Declarations

Ethical approval
Present study is approved by University Research Ethics Committee of DIT University, Dehradun. Informed consent form was taken from all volunteers for participation and publication of study outcome.

Consent to participate
The informed consent to participate in the study was obtained from the participants.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Faculty of Pharmacy, DIT University, Dehradun, Uttarakhand 248009, India. [2]Indira IVF Fertility Centre, First Floor, Ashirwad Towers, Ballupur Chowk, Chakrata Road, Dehradun, Uttarakhand 248001, India. [3]Morpheus Prasad International IVF Centre, Haridwar Bypass Rd, Opp. Suvidha Store, Maitri Vihar, Dehradun, Uttarakhand 248001, India.

Received: 9 November 2022 Accepted: 20 March 2023
Published online: 04 April 2023

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