A Subpathway-Based Method of Drug Reposition for Polycystic Ovary Syndrome

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Abstract

The need for development of new therapeutic agents for polycystic ovary syndrome (PCOS) is urgent due to general lack of efficient and specialized drugs currently available. We aimed to explore the metabolic mechanism of PCOS and inferred drug reposition for PCOS by a subpathway-based method. Using the GSE34526 microarray data from the Gene Expression Omnibus database, we first identified the differentially expressed genes (DEGs) between PCOS and normal samples. Then, we identified 13 significantly enriched metabolic subpathways that may be involved in the development of PCOS. Finally, by an integrated analysis of PCOS-involved subpathways and drug-affected subpathways, we identified 54 novel small molecular drugs capable to target the PCOS-involved subpathways. We also mapped the DEGs of PCOS and a potential novel drug (alprostadil) into purine metabolism pathway to illustrate the potentially active mechanism of alprostadil on PCOS. Candidate agents identified by our approach may provide insights into a novel therapy approach for PCOS.

Keywords

polycystic ovary syndrome, drug reposition, subpathway

Introduction

Polycystic ovary syndrome (PCOS) is an endocrine disorder associated with hyperandrogenism and chronic anovulation.¹ It affects female reproductive performance as well as it has effects on female health. It was originally described in 1935 by Stein IF and Leventhal ML as a syndrome consisting of oligomenorrhea and obesity with enlarged polycystic ovaries.¹ It leads to infertility in 4% to 12% of women of childbearing age.¹⁻³ The etiology of this syndrome is not well defined.¹ The clinical and biochemical features of PCOS appear to be heterogeneous.^{1,3-5} Polycystic ovary syndrome is often characterized by oligo- and/or anovulation, hyperandrogenism (clinical and/or biochemical), and ultrasonographic features (polycystic ovaries).^{2,4} The diagnosis of PCOS is often based on a combination of clinical, biological, and ultrasound criteria.⁶ There are several proposed diagnostic criteria for PCOS: National Institutes of Health (NIH) consensus criteria 1990, Rotterdam criteria 2003, and AES definition 2008.⁶ The NIH consensus criteria noted the disorder as having (1) hyperandrogenism and/or hyperandrogenemia, (2) oligoovulation, and (3) exclusion of known disorders.⁷ The AES definition focused on dealing with infertility management in PCOS.^{8,9} Currently, PCOS is defined by the 2003 Rotterdam criteria, which requires at least 2 of the 3 features for diagnosis: chronic anovulation, clinical and/or biochemical signs of hyperandrogenism, or polycystic ovaries.¹⁰ Polycystic ovary syndrome not only represents a major cause of infertility,

anovulation, hirsutism, and menstrual irregularities but also associates with metabolic abnormalities (insulin resistance) that may have severe influence for long-term health.^{1,3}

The therapeutic management of PCOS includes nonpharmacological approaches (weight reduction) and pharmacological approaches directed at the symptoms.^{3,11,12} For example, clomiphene is selected for inducing ovulation^{3,13}; spironolactone and eflornithine are first-line agents for hirsutism^{3,11,13}; and insulin-sensitizing agents, metformin (Glucophage), are indicated for most patients with PCOS.^{3,12} Although there has been notable progression in the development of agents for PCOS therapies, the cellular mechanisms of response to therapy are not elucidated enough^{1,3}; furthermore, the drugs for PCOS treatment are often directed at the different symptoms.^{1,3} Thus, special recommendations for treating PCOS are still lacking.^{1,3}

However, the de novo development of drugs is time consuming, costly, and risky. Therefore, drug repositioning,

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Table 1. The Top 10 Upregulated and Downregulated Genes.

Gene Symbol	Log2 (FC)	Function
CPXCR1	-3.751950078	Metal ion binding
NKX2-1	-3.421525695	DNA binding; RNA polymerase II distal enhancer sequence-specific DNA binding transcription factor activity; protein binding; sequence-specific DNA binding; sequence-specific DNA binding transcription factor activity; transcription regulatory region DNA binding
SIX3	-3.409118295	RNA polymerase II distal enhancer sequence-specific DNA binding transcription factor activity; sequence-specific DNA binding
MARVELD3	-3.313370508	Protein binding
PMP2	-3.298315492	Cholesterol binding; fatty acid binding; transporter activity
EFCC1	-3.284274499	Calcium ion binding
LOC100130285	-3.188537377	Unknown
CDK5R2	-3.182825521	Cyclin-dependent protein kinase 5 activator activity; lipid binding
GC	-3.172950199	Actin binding; calcidiol binding; vitamin D binding TAS; vitamin transporter activity
C17orf99	-3.069177776	protein coding
HLA-DRB4	9.520263391	MHC class II receptor activity; peptide antigen binding; protein binding
CLEC10A	5.887227288	Carbohydrate binding
HLA-DQB1	5.591375664	MHC class II receptor activity
CCL2	5.566336009	CCR2 chemokine receptor binding; CCR2 chemokine receptor binding; chemokine activity; heparin binding; protein kinase activity; receptor binding
SIGLEC1	5.410711633	Carbohydrate binding
EIF5A	5.347742161	RNA binding; U6 snRNA binding; poly(A) RNA binding; protein N-terminus binding; protein binding IPI; ribosome binding; translation elongation factor activity
RNASEI	5.337400968	Nucleic acid binding; pancreatic ribonuclease activity; protein binding
FAM20A	5.326174719	Protein coding
OLFM4	5.237608258	Cadherin binding; catalytic activity; protein homodimerization activity
CD52	5.232366476	Protein coding

Abbreviations: FC, fold change; snRNA, small nuclear RNA; CCR2, chemokine receptor type II.

namely, identifying new indication for known drugs, has become an effective and innovative approach in the development process, especially with the development of system biology and availability of public database.¹⁴ For example, Gene Expression Omnibus (GEO) database and CMap database had provided numerous microarray data sets under disease or drug-induced conditions.¹⁵ Here, we presented a computational method for drug repositioning of PCOS based on metabolic subpathway analysis. We first explored the metabolic mechanism of PCOS and then identified known small molecular drugs capable of targeting the metabolic subpathways that were dysregulated in the development of PCOS. Although further evaluations are still needed, the candidate agents identified by our approach may provide great insights into improving therapeutic status for PCOS.

Results

Analysis of Differentially Expressed Genes Between Patients With PCOS and Healthy Controls

In order to explore the mechanism of PCOS, we obtained the expression profiles of PCOS samples and normal samples from GEO database (<http://www.ncbi.nlm.nih.gov/geo/>). We obtained the available microarray data set GSE34526 from the GEO database and then used fold change (FC) method to identify the differentially expressed genes (DEGs) between patients with PCOS and controls.¹⁶ A total of 7027 genes were considered differentially expressed (see Methods and Materials

section). All DEGs and corresponding FC values are shown in supplementary data set 1. The top 10 upregulated and down-regulated genes are listed in Table 1. To explore the biological functions of DEGs, we conducted Gene Ontology Biological Process functional enrichment analysis of the DEGs using the database for annotation, visualization, and integrated discovery (DAVID) tool¹⁷ (<http://david.abcc.ncifcrf.gov/>). The statistically significant results are shown in supplementary data set 2 (P value < .01).

Exploring Mechanism of PCOS Based on Subpathway Enrichment Analysis

To explore mechanism of PCOS, we identified dysregulated metabolic subpathways using Subpathwayminer (<http://cran.r-project.org/src/contrib/Archive/SubpathwayMiner/>).¹⁸ We annotated the DEGs to entire metabolic pathways and subpathways ($k = 3$). With the strict cutoff of P value less than .01, we identified 13 enriched metabolic subpathways corresponding to 5 entire metabolic pathways (Table 2). These 13 metabolic subpathways have cross-talks in the corresponding entire pathways and were significantly related to development of PCOS.

Drug Reposition for PCOS

To identifying candidate small molecular drugs that were capable to target PCOS-related metabolic subpathways, we

Table 2. The Enriched Subpathways and Corresponding Entire Pathways.

Entire Pathway ID	Pathway Name	Subpathway ID	P Value	Number of Genes
path:00562	Inositol phosphate metabolism	path:00562_6	.000387	23
path:00760	Nicotinate and nicotinamide metabolism	path:00760_6	.000438	20
	Nicotinate and nicotinamide metabolism	path:00760_10	.000438	20
	Nicotinate and nicotinamide metabolism	path:00760_7	.001153	15
path:00230	Purine metabolism	path:00230_1	.003971	26
	Purine metabolism	path:00230_9	.006096	28
	Purine metabolism	path:00230_16	.006096	28
	Purine metabolism	path:00230_7	.006656	45
	Purine metabolism	path:00230_8	.009889	52
path:00240	Pyrimidine metabolism	path:00240_8	.001414	19
	Pyrimidine metabolism	path:00240_5	.003382	17
	Pyrimidine metabolism	path:00240_1	.00915	5
path:00500	Starch and sucrose metabolism	path:00500_9	.006531	14

downloaded the global associations between drugs and metabolic subpathways from the article of Li et al, in which we identified 403 metabolic subpathways affected by 488 known drugs by also using Subpathwayminer with $k = 3$ (see Methods and Materials section).¹⁹

By integrating the 13 PCOS-related subpathways and these 403 drug-induced subpathways, we selected the overlapping subpathways that are related to both PCOS and small molecular drugs. Thus, we could assume that the small molecular drugs that affected the overlapping subpathway might play a role in perturbing the development of PCOS. A total of 54 small molecular drugs were identified (Table 3).

We then built a network between these repositioned drugs and the overlapping metabolic subpathways by integrating the relationships mentioned earlier (Figure 1). In this network, some small drugs could perturb many metabolic subpathways (eg, pergolide and alprostadil perturbed 7 and 6 subpathways), respectively, while some small molecular drugs could perturb only a few subpathways (Figure 1).

Pathway Annotation of DEGs of Alprostadil and PCOS in Purine Metabolism

To elucidate the potential involvement of alprostadil in PCOS in detail, we mapped the DEGs of PCOS and alprostadil into the pathway of purine metabolism (path:00230) using the “user data mapping” tool in Kyoto Encyclopedia of Genes and Genomes (KEGG) database (<http://www.genome.jp/kegg/>). The DEGs of alprostadil were downloaded from the supplementary data sets in studies of Li et al.¹⁹ The results are shown in Figure 2. We found that many enzymes were perturbed by both alprostadil (red pentagram) and PCOS (red rectangle), including phosphodiesterase (PDE; blue circle).

Discussion

Analysis of disease gene expression profiling could help us reveal the underlying mechanism of complex diseases and provide us potential targets for improving therapeutic intervention.

In this research, we used the gene expression profile downloaded from GEO to explore the underlying mechanism of development of PCOS. This gene expression profile was based on human granulosa cells (GCs) which was anatomically proximal to the ovarian germ cells. Their functional communication with oocytes was not only essential for oocyte and follicular growth but also crucial for fertility.²⁰ A total of 7027 DEGs were identified between PCOS samples and healthy controls. Subpathway mining result showed that 13 metabolic subpathways corresponding to 5 entire pathways were dysfunctional in PCOS. Then, we identified the potential therapeutic agents for PCOS using a computational drug reposition method. A total of 54 small molecules were identified, which may play a role in perturbing the development of PCOS.

Currently, majority of pathway-enrichment approaches commonly focus on the entire pathways.¹⁸ However, analysis of subpathways, which focuses on certain local areas of pathways, can identify more subtle subpathways that are neglected by entire pathways analysis. It may be more suitable and flexible than entire pathways for identification of disease mechanism and drug responses.^{18,19} In this study, we identified 13 subpathways from 5 KEGG metabolic pathways. On one hand, some of these subpathways have well-documented metabolic and hormonal effects in PCOS. For example, the most significant subpathway, inositol phosphate metabolism (path:00562_6; P value = .000387), has been reported to be involved in the pathogenesis of insulin resistance and administration of myo-inositol (MYO) has demonstrated to improve the insulin receptor activity for potentially restoring spontaneous ovulatory function in most of women with PCOS.^{2,21,22} Another example is starch and sucrose metabolism (path:00500_9; P value = .006531). The metabolism of sucrose is found to be impaired in ovarian capsule with polycystic ovarian disease, resulting in lower level of androgen-binding protein compared with normal ovarian capsule.²³ In this subpathway, some DEGs have been reported to associate with PCOS. For example, GYS2 ($FC = 2.5$) was identified as a novel genetic factor for PCOS by a genome-wide association study.²⁴ On the other hand, the roles of other pathways in PCOS have not been well defined previously. But some key enzymes or

Table 3. Potential Novel Drugs and Their Information.^a

Drug ID	Drug Name	Description	Number of Overlaps	ATC Code
DB01186	Pergolide	Dopamine agonist	7	N04BC02
DB00770	Alprostadil	Prostaglandins	6	C01EA010
D07442	Ambroxol	Mucolytics	4	R05CB06
DB01190	Clindamycin	Anti-infectives for treatment of acne	4	D10AF01
D00081	Dinoprost	Prostaglandins	4	G02AD01
DB00714	Apomorphine	Dopamine D2 agonist	3	G04BE07
D07926	Ethaverine	Antispasmodic	3	N
DB04570	Lamoxef	Third-generation cephalosporins	3	J01DD06
DB00816	Orciprenaline	β -adrenergic agonist	3	R03CB03
DB01667	8-Azaguanine	Antineoplastic activity	2	L
DB01426	Ajmaline	Antiarrhythmics, class Ia	2	C01BA05
DB00479	Amikacin	Semisynthetic aminoglycoside antibiotic	2	D06AX12
D07192	Benfluorex	Other blood glucose-lowering drugs, excl. insulins	2	A10BX06
DB00878	Chlorhexidine	Anti-infectives and antiseptics for local oral treatment	2	A01AB03
D07689	Chlortetracycline	Anti-infectives and antiseptics for local oral treatment	2	A01AB21
DB00356	Chlorzoxazone	Oxazol, thiazine, and triazine derivatives	2	M03BB03
D03625	Cicloheximide	Antipsoriatic	2	D
DB00501	Cimetidine	H ₂ -receptor antagonists	2	A02BA01
DB01013	Clobetasol	Corticosteroids, very potent (group iv)	2	D07AD01
DB01406	Danazol	Antigonadotropins and similar agents	2	G03XA01
DB00746	Deferoxamine	Iron chelating agents	2	V03AC01
DB00343	Diltiazem	Benzothiazepine derivatives	2	C08DB01
DB00687	Fludrocortisone	Mineralocorticoids	2	H02AA02
DB01016	Glibenclamide	Sulfonamides, urea derivatives	2	A10BB01
DB00762	Irinotecan	Other antineoplastic agents	2	L01XX19
D02364	Lobeline	Respiratory stimulant	2	R
DB00253	Medrysone	Corticosteroids, plain	2	S01BA08
DB00916	Metronidazole	Anti-infectives and antiseptics for local oral treatment	2	A01AB17
D01339	Midecamycin	Macrolides	2	J01FA03
DB01183	Naloxone	Antidotes	2	V03AB15
D00862	Nitrofurantoin	Anti-infectives	2	B05CA03
DB01229	Paclitaxel	Taxanes	2	L01CD01
D05653	Puromycin	Antineoplastic; antiprotozoal	2	J
DB01361	Troleandomycin	Macrolides	2	J01FA08
DB00313	Valproic acid	Fatty acid derivatives	2	N03AG01
D01545	Aceclofenac	Acetic acid derivatives and related substances	1	M01AB16
DB00414	Acetohexamide	Sulfonamides, urea derivatives	1	A10BB31
D07125	Alimemazine	Phenothiazine derivatives	1	R06AD01
D07576	Asiaticoside	Antioxidation	1	D
DB00928	Azacitidine	Pyrimidine analogs	1	L01BC07
DB01327	Cefazolin	First-generation cephalosporins	1	J01DB04
D01334	Ciclacillin	Antibacterial	1	J
D03715	Dexibuprofen	Propionic acid derivatives	1	M01AE14
DB02187	Equilin	Estrogen	1	G
D00540	Glycopyrronium bromide	Synthetic anticholinergics, quaternary ammonium compounds	1	A03AB02
D04625	Isoetarine	Selective β -2-adrenoreceptor agonists	1	R03AC07
D01972	Lanatoside c	Digitalis glycosides	1	C01AA06
DB00563	Methotrexate	Folic acid analogs	1	L01BA01
D01320	Molsidomine	Other vasodilators used in cardiac diseases	1	C01DX12
DB04552	Niflumic acid	Anti-inflammatory preparations, nonsteroids for topical use	1	M02AA17
D01379	Proscillaridin	Scilla glycosides	1	C01AB01
DB00576	Sulfamethizole	Anti-infectives	1	B05CA04
DB00580	Valdecoxib	Coxibs	1	M01AH03
DB02546	Vorinostat	Other antineoplastic agents	1	L01XX38

^a ATC codes are according to "The Anatomical Therapeutic Chemical (ATC) Classification System" (<http://www.whocc.no/>).

metabolites were found to be related to PCOS. For example, NAD(P)H, a metabolite in nicotinate and nicotinamide metabolism (path:00760), was found to have a higher level in oocytes of

patients with PCOS.²⁵ We also note that 5 of 13 enriched sub-pathways belonged to the entire pathway of purine metabolism (path:00230), which was not found to have direct relation to

Figure 1. Small molecular drugs and their perturbed subpathways in polycystic ovary syndrome (PCOS). The triangular nodes present subpathways and the circular nodes represent drugs. The subpathways in the same color are included in the same entire pathways. The description of the corresponding entire pathways is shown on the left part. (The color version of this article is available at <http://rs.sagepub.com>.)

PCOS previously. But it is reported that some key enzymes (ie, adenosine deaminase) of purine metabolism are commonly associated to bioactivity of insulin and play a contributory role in patients with insulin resistance which appears in majority of women with PCOS.²⁶

We then identify a group of known drugs with potential novel therapeutic efficacy for PCOS. A total of 54 small molecules, which have common subpathways with PCOS, were identified including pergolide (number of overlaps = 7) and alprostadil (number of overlaps = 6). Pergolide, an ergot-derived dopamine D₂ receptor agonist, is used for pituitary pars intermedia dysfunction.²⁷ Although we could not find the direct reports of pergolide involvement in PCOS, it is related to insulin resistance and used to treat type 2 diabetes mellitus in animal models. Alprostadil, known pharmaceutically as prostaglandin E₁ (PGE₁), has vasodilatory properties and is used widely in various clinical settings, and prostaglandins produced by ovarian GCs are essential for ovulation.^{28,29} Although there are a few reports about the associations between PGE₁ and PCOS, alprostadil has not been used as a therapeutic management for PCOS clinically, and the underlying mechanism is not yet clear. From Figure 1, we find that 5 of 6 overlap subpathways belong to the “Purine metabolism” (path:00230). For detailed elucidation of potential involvement of alprostadil in PCOS, we mapped the DEGs of PCOS and alprostadil into

the pathway of purine metabolism (path:00230; Figure 2). We found that lots of PCOS-involved genes are also perturbed by alprostadil. Interestingly, of these overlapped genes, PDEs (blue circle in Figure 2) are found to have genetic variation in PCOS and it participates in the cyclic adenosine monophosphate (cAMP) degradation, while cAMP can be produced by adenylate cyclase after activation by PGE₁.^{30,31} These findings indicate potential therapeutic targets for PCOS.

We still note that there were some limitations in our studies. There were only small molecular drugs in our studies. The scope of candidate drugs will be expanded by integrating more drug-affected data from different resources. Also, further experimental verification and population-based studies are still needed to make these agents safely available clinically. Although our method and data are far from completeness, our study still presents a bioinformatics approach based on subpathway analysis to infer drug reposition for PCOS and may provide insights into a novel therapy approach for PCOS.

Methods and Materials

Microarray Data and DEGs Analysis

The microarray data of GSE34526 was obtained from GEO database (<http://www.ncbi.nlm.nih.gov/geo/>) in the National

Figure 2. The enzymes perturbed by polycystic ovary syndrome (PCOS) and alprostadil. The enzymes perturbed by PCOS and alprostadil are marked by red rectangle and red pentagram, respectively. The blue circle presents phosphodiesterase (PDE). (The color version of this article is available at <http://rs.sagepub.com>.)

Center of Biotechnology Information.³² This profile was based on human GCs isolated from ovarian aspirates of normal women and women with PCOS.¹⁶ A total of 7 patients with PCOS and 3 normal women were profiled using the Affymetrix Human Genome U133 Plus 2.0 Array (HG-U133_Plus_2) platform.¹⁶

We downloaded the raw microarray data and the probe annotation files for further analysis. At first, we calculated the FC values for each probes. Then, we converted each probes into gene Entrez Gene IDs. If 1 gene matched more than 1 probes, the expression value of this gene was computed by taking the average expression value of all corresponding probes. Finally, the genes with FC value more than 2 or less than 0.5 were identified as DEGs.

Obtainment of Subpathways by SubpathwayMiner

To obtain the perturbed subpathway under PCOS, we used the SubpathwayMiner software package which is a flexible subpathway identification method.¹⁸ In this software, the pathway structure data with KGML format provided by KEGG is converted to R graph objects.³³ Then, the enzymes are considered as nodes and 2 nodes in an undirected graph are connected by an edge if there is a common compound in the reactions of the corresponding enzymes. This software package can transform the entire pathway into subpathways by “k-clique” method.¹⁸ In social network analysis, a k-clique in a graph is defined as a subgraph in which the distance between any 2 nodes is no greater than a parameter, k.¹⁸ Here, we set $k = 3$, and this parameter setting means that the distance among enzymes in 1 subpathway is not greater than 3.

We then imported the DEGs (from microarray data of GSE34526) into SubpathwayMiner, and this software could identify significantly enriched subpathways. The P value less than .01 is chosen as the cutoff criterion for statistically significant subpathways.

The Associations Between Drugs and Metabolic Subpathways

To find the novel drugs that may play a role in the PCOS-involved subpathways, we obtained the global associations between drugs and metabolic subpathways from the article of Li et al.¹⁹ They first downloaded microarray data for each small molecular drug from the CMap database and identified the DEGs for each drug by a cutoff with $|FC| > 1$ (after logarithmic transformation).¹⁵ Then, they identified drug-affected subpathways for each drug and whether the corresponding drug-affected genes could be significantly enriched to the subpathways by the SubpathwayMiner software package (P value $< .01$).¹⁹ Finally, this article obtained 403 metabolic subpathways corresponding to 488 drugs.

Identification of Potential Drugs for PCOS

In the article of Li et al, they identified 403 drug-related metabolic subpathways.¹⁹ In this article, we identified 13

PCOS-related metabolic subpathways. The overlaps of the above-mentioned 2 sets of metabolic subpathways were selected. We found that all 13 PCOS-related subpathways were included in 403 drug-related subpathways. These 13 overlapping subpathways were not only drug affected but also involved in the development of PCOS. They were corresponding to 54 drugs according to the drug–metabolic subpathway associations in the article of Li et al.¹⁹

Declaration of Conflicting Interests

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Supplemental Material

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