A Systems Biology Approach for Investigating Significant Biomarkers and Drug Targets Common Among Patients with Gonorrhea, Chlamydia, and Prostate Cancer: A Pilot Study

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ABSTRACT: Having a previous history of sexually transmitted diseases (STDs) such as gonorrhea and chlamydia increases the chance of developing prostate cancer, the second most frequent malignant cancer among men. However, the molecular functions that cause the development of prostate cancer in persons with gonorrhea and chlamydia are yet unknown. In this study, we studied RNA-seq gene expression profiles using computational biology methods to find out potential biomarkers that could help us in understanding the patho-biological mechanisms of gonorrhea, chlamydia, and prostate cancer. Using statistical methods on the Gene Expression Omnibus (GEO) data sets, it was found that a total of 22 distinct differentially expressed genes were shared among these 3 diseases of which 14 were up-regulated (PGRMC1, TSC22D1, SH3BGRL, NNT, CTSC, FRMD3, CCR2, FAM210B, VCL, PTGS1, SLFN11, SLC40A1, PROS1, and DSE) and the remaining 8 genes were down-regulated (PRNP, HINT3, MARCKSL1, TMED10, SH3KBP1, ENSA, DERL1, and KMT2B). Investigation on these 22 unique dysregulated genes using Gene Ontology, BioCarta, KEGG, and Reactome revealed multiple altered molecular pathways, including regulation of amyloid precursor protein catabolic process, ferroptosis, effects on gene expression of *Homo sapiens* PPAR pathway, and innate immune system R-HSA-168249. Four significant hub proteins namely VCL, SH3KBP1, PRNP, and PGRMC1 were revealed by protein-protein interaction network analysis. By analyzing gene-transcription factors and gene-miRNAs interactions, significant transcription factors (POU2F2, POU2F1, GATA6, and HIVEP1) and posttranscriptional regulator microRNAs (hsa-miR-7-5p) were also identified. Three potential therapeutic compounds namely INCB3284, CCX915, and MLN-1202 were found to interact with up-regulated protein C-C chemokine receptor type 2 (CCR2) in protein-drug interaction analysis. The proposed biomarkers and therapeutic potential molecules could be investigated for potential pharmacological targets and activity in the fight against in patients with gonorrhea, chlamydia, and prostate cancer.

KEYWORDS: Prostate cancer, gonorrhea, chlamydia, STD, biomarker identification, drug candidate.

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Introduction

Gonorrhea is a widely known sexually transmitted disease (STD) all over the world which is caused by a bacterium named *Neisseria gonorrhoeae* that infects the mucosa of exposed anatomic areas, including urogenital tract, rectum, mouth, and conjunctiva. Without proper treatment, cervical gonorrhea can have serious effects on reproductive health.[1,2] The symptoms in females include abnormal uterine bleeding, vaginal discharge, dyspareunia, dysuria, lower abdominal and/or rectal pain while in male dysuria, urethral discharge and/or itching, and testicular or rectal pain are common. The urethra and cervix, followed by the anal and pharyngeal regions, are the anatomical sites that are the most frequently impacted.[3] From roughly 13 cases per 100 people in 2008 to up to 29 cases per 100 people in 2017, the Public Health Agency of Canada (PHAC) reported that the prevalence of gonorrhea has more than doubled since 2013.[4] The reported rate in the United States seems to be much higher with 171.9 cases per 100 000 people in 2017.[5] World Health Organization (WHO) reported 82 million new infections of gonorrhea in 2022 alone.[6]

The infection of a bacterium *Chlamydia trachomatis* results in STD named chlamydia. In females, urethritis, cervicitis, pelvic inflammatory disease, proctitis, and perihepatitis are some of the symptoms of this disease and if proper treatment is not received in a timely manner, it might result in infertility and ectopic pregnancy. In case of men, the bacterium can cause epididymitis, urethritis, proctitis, prostatitis, or reactive arthritis.[7] Besides, this pathogen is the most common preventable cause of blindness in the endemic regions such as Africa and the Middle East.[8] According to Centers for Disease Control and Prevention (CDC) in 2018, almost 4 million people are diagnosed with chlamydia in the United States.[9] According to WHO, 129 million new infections of chlamydia were reported in 2022 alone.[6] The PHAC, Canada, reported a national rate of 345.7 cases of chlamydia per 100 000 populations in 2017.[4] In men, the symptoms of chlamydia include pain in testicles, burning sensation during urination, yellow or green discharge from the urinating part, pain in lower abdomen while in case of women, painful feeling during sexual intercourse, vaginal discharge, inflammation of cervix, burning sensation when

urinating and most importantly bleeding between menstrual cycles are observed.[10]

Prostate cancer is the second most frequent malignant cancer among men. According to the American Cancer Society, about 288 300 new cases were reported and about 34 700 people died from prostate cancer in 2023.[11] It was stated that this cancer affects more than 35 000 men in the United Kingdom alone every year, and 10 000 men pass away as a result of the condition. Although the patient may be asymptomatic, the main complications with this disease are micturition, straining to start, frequency, and nocturia.[12] It is evident that access to diagnosis and treatment affects prostate cancer rates significantly. Males of African heritage have greater prostate cancer rates and lower prognosis.[13]

Having a previous history of STDs like chlamydia or gonorrhea may increase the possibility of developing prostate cancer. A research funded by California Cancer Research Program, and Community Benefits Program of Kaiser Permanente found that 26.3% of males reported a history of any STD, with 14.7% of those men having experienced 2 or more STDs before being diagnosed with prostate cancer. The most prevalent STD among them was gonorrhea (17.1%), and the percentage of chlamydia was 3.6%.[14] Another study related to chlamydia shows that, in human prostate epithelial cells, the expression of inflammatory tumorigenic cytokines and chemokines is promoted by the infection of *C. trachomatis*, including the components of the Toll-like receptor and NF-kB pathways. According to this research, epithelial cells from human prostate cancer are prone to *C. trachomatis* infection and increase more pro-inflammatory markers when infected.[15] Although it is well-thought-out that history of previous STDs like chlamydia, and gonorrhea may impact the development of prostate cancer, the markers which are often dysregulated in those 3 diseases have not been well considered at molecular levels till now using RNA data sets and microarray data which are available on online databases like NCBI.

In our research, a system biology approach has been employed to reveal differentially expressed genes (DEGs) and related molecular pathways identical among patients with gonorrhea, chlamydia, and prostate cancer. The DEGs shared among the 3 diseases were studied in interaction networks using the following tactics: (1) study of gene enrichment to determine cellular components, molecular functions, and biological processes in which a gene is associated, (2) finding hub genes using the protein-protein interaction (PPI) network, (3) identification of transcription factors (TFs) and miRNAs that are associated with the common DEGs, and (4) promising drug candidates screening using protein-drug interaction networks. The scientific findings of this study will aid in revealing biomarkers for disease diagnosis and molecular targets for drug development which will be used to treat these diseases.

Methods

For biomarkers and drug target identification, data from NCBI-Gene Expression Omnibus (GEO) database were used and statistical approaches were applied to select shared dysregulated genes among the 3 disorders, namely gonorrhea, chlamydia, and prostate cancer. Overall procedures are illustrated in Figure 1.

Microarray data sets

This study used data from the NCBI-GEO database, GSE110106, GSE180238, and GSE38241 with a total sample size of 49. The 4 samples from GSE110106 come from 2 healthy individuals (GSM2977717 and GSM2977718) and 2 gonorrhea affected individuals with biopsy-confirmed *N gonorrhoeae* (GSM3093828 and GSM3093832). In case of chlamydia, there were 6 individuals with 3 patients and 3 healthy come from GSE180238 where *C. trachomatis*-infected human leukaemia (HL-60) samples were considered as patient and uninfected HL-60 samples were considered as control. For prostate cancer, 39 samples come from GSE38241 with 18 patients and 21 healthy individuals where normal prostate from organ donors considered as control and samples from lethal metastatic prostate cancer considered as patient. The control groups, which served as a reference in the data sets, didn't show any inflammation.

Analysis of differential gene expression

Differentially expressed genes were recognized using GEO2R by comparing patients to their respective control groups in all 3 GEO data sets. Statistically significant DEGs were identified by considering *log2 (Fold Change) ⩾0.5 for up-regulated genes and log2 (Fold Change) ⩽−0.5 for down-regulated genes* with a P value less than .05 and the Benjamini and Hochberg (false-discovery rate) criterion for P value adjustment. The Venny tool was used to find DEGs that were common among gonorrhea, chlamydia, and prostate cancer (https://bioinfogp.cnb.csic.es/tools/venny/).

Gene ontology and DEG pathway enrichment

To learn more about the DEGs, gene enrichment analysis was carried out. Enrichr (https://maayanlab.cloud/Enrichr/) was used to obtain the cellular components, molecular activities, and biological processes to which a gene contributes using $P < .05$ and also common pathways among gonorrhea, chlamydia, and prostate cancer were determined through KEGG, Reactome, and BioCarta pathways (https://maayanlab.cloud/Enrichr/).

PPI network study

Protein-protein interaction networks were created using STRING protein interaction database, and Network Analyst

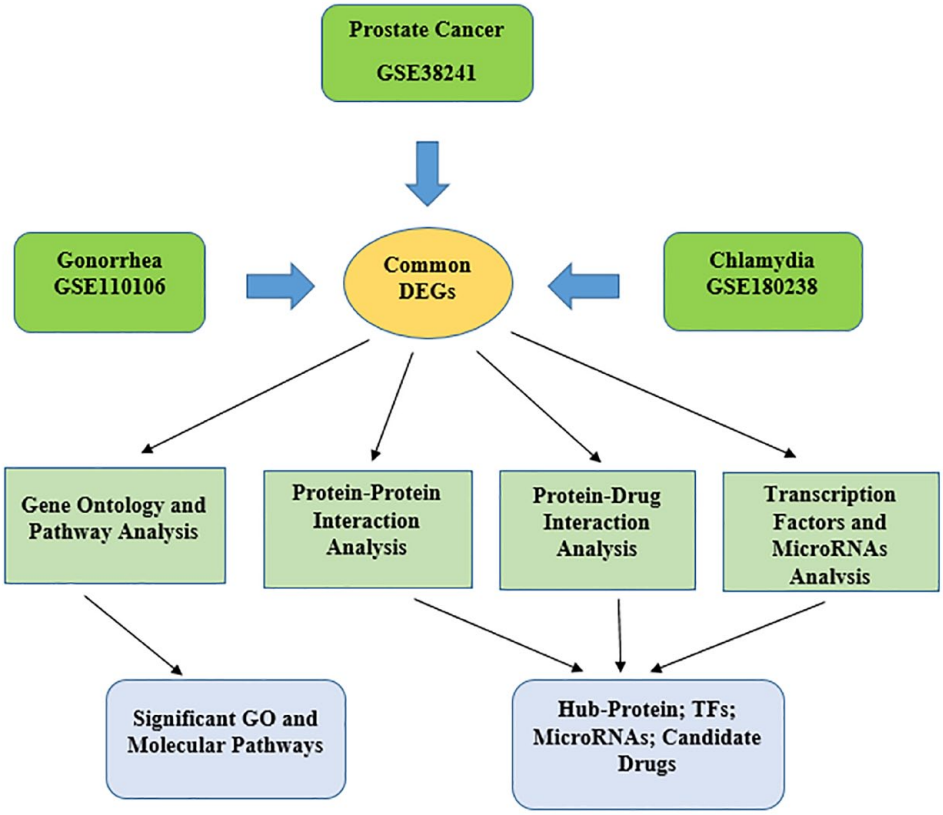

Figure 1. The entire methodology employed in this study. Differentially expressed genes for gonorrhea, chlamydia, and prostate cancer were identified, and then, statistical approaches were applied to select shared dysregulated genes among these 3 disorders. To identify important shared pathways and GO keywords, gene enrichment analysis was carried out. PPI analysis was performed to identify hub proteins, TFs, and miRNAs that regulate those DEGs. Finally, protein-drug interactions were used to identify potential therapeutic candidates.
DEGs indicates differentially expressed genes; miRNAs, microRNA; GO, gene ontology; PPI, protein-protein interaction.

web resource, with a confidence score of 700 (https://www.networkanalyst.ca/).

Investigation of DEG interaction with transcriptional and posttranscriptional regulators

Using TRANSFAC, and miRTarBase databases, prominent transcription factors and microRNAs that regulate DEGs of importance at both the transcriptional and posttranscriptional levels, respectively, were mined with a P value $< .05$ (https://maayanlab.cloud/Enrichr/). Top 10 miRNAs were selected, and their interactions with target genes were validated using RNAhybrid tool (https://bibiserv.cebitec.uni-bielefeld.de/rnahybrid) to calculate minimum free energy on hybridization with target mRNA.

Assessment of protein–drug interactions

Effective therapeutic candidates for gonorrhea, chlamydia, and prostate cancer were revealed by analyzing protein-drug interactions with Network Analyst (https://www.networkanalyst.ca/) tool with the help of Drug Bank database (version 5.0).

Results

Identification of DEGs shared among gonorrhea, chlamydia, and prostate cancer patients

Gene expressions of microarray data sets were studied for gonorrhea, chlamydia, and prostate cancer, and significant DEGs were identified using statistical methods. It was found that a total of 22 distinct genes were discovered to be shared among the gonorrhea DEGs, chlamydia DEGs, and prostate cancer DEGs of which 14 were up-regulated (PGRMC1, TSC22D1, SH3BGRL, NNT, CTSC, FRMD3, CCR2, FAM210B, VCL, PTGS1, SLFN11, SLC40A1, PROS1, and DSE). The remaining 8 genes were down-regulated (PRNP, HINT3, MARCKSL1, TMED10, SH3KBP1, ENSA, DERL1, and KMT2B) (Figures 2 and 3).

These shared 22 DEGs were chosen for gene set enrichment analysis to understand more about the biological processes, molecular functions, and cellular components that a gene associates. The enriched biological processes are regulation of amyloid precursor protein catabolic process, regulation of amyloid-beta formation, protein destabilization, positive regulation of lymphocyte activation, regulated exocytosis, ER to Golgi vesicle-mediated transport, and others.

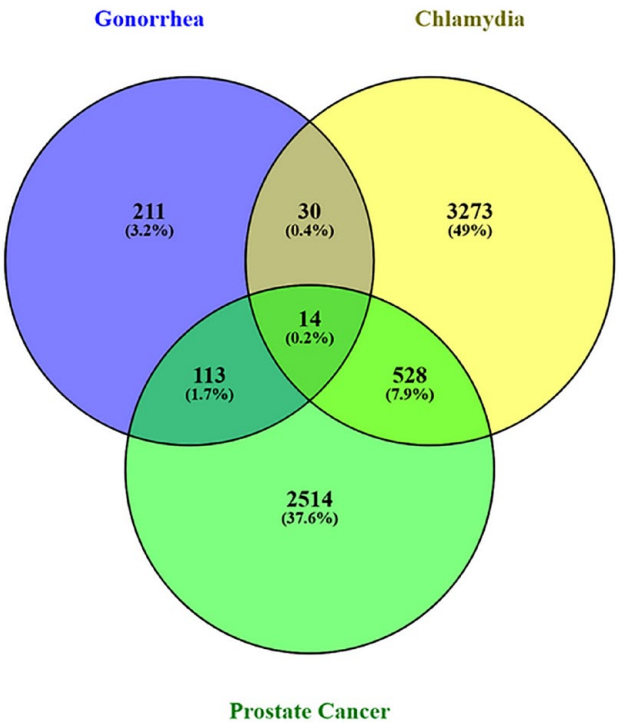

Figure 2. The Venny tool (www.bioinfogp.cnb.csic.es/tools/venny/) was used to identify genes that are commonly up-regulated in gonorrhea, chlamydia, and prostate cancer. In gonorrhea, chlamydia, and prostate cancer, a total of 14 genes were discovered to be frequently up-regulated.

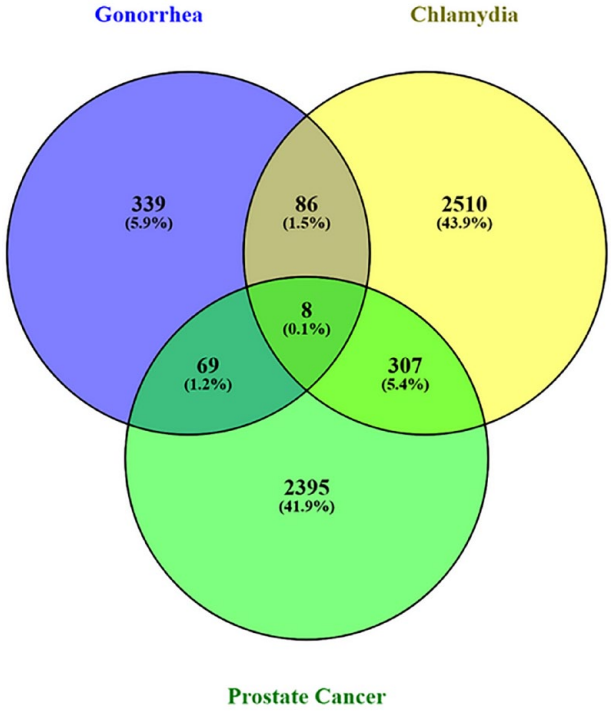

Figure 3. The Venny tool (www.bioinfogp.cnb.csic.es/tools/venny/) was used to identify genes that are commonly down-regulated in gonorrhea, chlamydia, and prostate cancer. A total of 8 genes were found to be frequently down-regulated in gonorrhea, chlamydia, and prostate cancer.

The significant molecular functions of DEGs shared by gonorrhea, chlamydia, and prostate cancer were amyloid-beta binding, endopeptidase inhibitor activity, protease binding, signal recognition particle binding, and others, and the important cellular components were found as zymogen granule ER-Golgi intermediate compartment membrane, COPII-coated ER to Golgi transport vesicle coated vesicle, secretory granule lumen, and others as shown in Table 1. Common pathways are identified by KEGG. BioCarta and Reactome are summarized in Table 2.

Hub protein identification from PPI network

The PPI network generated by STRING consists of 4 hub nodes from shared DEGs and 187 edges (Figure 4) that uncovered 4 hub proteins, namely VCL, SH3KBP1, PRNP, and PGRMC1 (Table 3). Hub proteins were selected based on the higher number of connectivity with shared DEGs in the PPI network (degree of connectivity >10).

Revealing transcriptional and posttranscriptional regulators that interact with DEGs related to prostate cancer, gonorrhea, and chlamydia patient

Because the expression of genes is regulated at both the transcriptional and posttranscriptional levels, significant transcription factors and miRNAs that regulate expression of shared DEGs among gonorrhea, chlamydia, and prostate cancer were determined. We found significant TFs POU2F2 (targeting DEG PRNP, SH3BGRL, TMED10, TSC22D1, HINT3, PROS1, DSE, and CTSC), POU2F1 (targeting DEG PRNP, SH3BGRL, TMED10, PROS1, and DSE), GATA6 (targeting DEG TMED10, TSC22D1, ENSA, DERL1, and CCR2), and HIVEP1 (targeting DEG FRMD3 and TSC22D1) (Table 3). In addition, the top 10 statistically prominent miRNAs were found namely hsa-miR-1224-3p, hsa-miR-485-3p, hsa-miR-125a-5p, hsa-miR-7-5p, hsa-miR-5572, hsa-miR-4717-5p, hsa-miR-153-3p, hsa-miR-506-5p, hsa-miR-4518, and hsa-miR-1266-5p are presented in Table 4 of which all showed negative free energy change on binding with target mRNA indicating possibility of interaction and gene regulation.

Effective therapeutics identification by protein–drug interaction network

C-C chemokine receptor type 2 gene encodes a protein which acts as a functional receptor (C-C motif chemokine receptor 2) for CCL2 but also can bind with CC7 and CCL12.[16-18] It has been reported that polymorphisms in CCR and its ligand monocyte chemoattractant protein-1 (MCP-1) are associated with various diseases.[19-23] Several research reported CCR2 protein's involvement with prostate cancer.[24,25]

As this CCR2 gene is up-regulated in gonorrhea, chlamydia, and prostate cancer, our objective was to reduce the

Table 1. Significant gene ontology (GO) terms related to common differentially expressed genes in gonorrhea, chlamydia, and prostate cancer.

GROUP	GO TERM	*P*
Biological process	Regulation of amyloid precursor protein catabolic process	.000076
	Regulation of amyloid-beta formation	.000634
	Protein destabilization	.000634
	Positive regulation of lymphocyte activation	.000672
	Regulated exocytosis	.000973
	Endoplasmic reticulum to Golgi vesicle-mediated transport	.001053
	Regulation of interleukin-2 production	.001263
	COPII vesicle coating	.002166
	Vesicle coating	.002166
	Vesicle targeting, rough endoplasmic reticulum (ER) to cis-Golgi	.002166
Molecular function	Amyloid-beta binding	.003465
	Endopeptidase inhibitor activity	.007023
	Protease binding	.007381
	Aspartic-type endopeptidase inhibitor activity	.007676
	Signal recognition particle binding	.007676
	Cuprous ion binding	.008768
	G protein-coupled glutamate receptor binding	.008768
	Iron ion transmembrane transporter activity	.008768
	Unmethylated CpG binding	.008768
	Potassium channel inhibitor activity	.009858
Cellular component	Endoplasmic reticulum-Golgi intermediate compartment membrane	.001316
	COPII-coated endoplasmic reticulum (ER) to Golgi transport vesicle	.003380
	Coated vesicle	.003813
	Secretory granule lumen	.004816
	Zymogen granule	.006582
	Zymogen granule membrane	.006582
	Endoplasmic reticulum membrane	.006986
	Gamma-secretase complex	.007676
	COPI-coated vesicle	.007676
	Mitochondrial outer membrane	.008376

$P < .05$.

expression of this gene when it binds with a potential therapeutic substances. By using Network Analyst tool, drug bank database search against DEGs found existing drugs namely INCB3284, CCX915, and MLN-1202 interactions with commonly up-regulated gene CCR2 (Table 5 and Figure 5). According to drug bank, INCB3284 is an antagonist for CCR2 and currently being investigated to be used inflammatory disorder treatment while CCX315 is in phase 1 of clinical trial for the treatment of multiple sclerosis and neurologic disorders. MLN-1202 is a humanized monoclonal antibody which has been investigated for the treatment of atherosclerosis.

Table 2. Genes that are variably expressed and are common among gonorrhea, chlamydia, and prostate cancer patients contribute to various molecular pathways ($P < .05$).

PATHWAYS	*P*	GENES INVOLVED IN PATHWAYS
KEGG (Kyoto Encyclopedia of Genes and Genomes)		
Ferroptosis	9.23E-04	PRNP and SLC40A1
Nicotinate and nicotinamide metabolism	.037820	NNT
BioCarta		
Basic mechanism of action of PPARa, PPARb(d), and PPARg and effects on gene expression Homo sapiens h PPAR pathway	.005488	PTGS1
Mechanism of acetaminophen activity and toxicity Homo sapiens h acetaminophen pathway	.007676	PTGS1
CBL mediated ligand-induced downregulation of EGF receptors Homo sapiens h cbl pathway	.008768	SH3KBP1
Cell-to-cell adhesion signaling Homo sapiens h cell-to-cell pathway	.013124	VCL
Extrinsic prothrombin activation pathway Homo sapiens h extrinsic pathway	.014210	PROS1
Aspirin blocks signaling pathway involved in platelet activation Homo sapiens h spaa pathway	.018544	PTGS1
Prion pathway Homo sapiens h prion pathway	.019624	PRNP
Sprouty regulation of tyrosine kinase signals Homo sapiens h spry pathway	.021782	SH3KBP1
Eicosanoid metabolism Homo sapiens h eicosanoid pathway	.025009	PTGS1
Intrinsic prothrombin activation pathway Homo sapiens h intrinsic pathway	.025009	PROS1
Reactome		
Innate immune system R-HSA-168249	.004629	PGRMC1, PROS1, CTSC, VCL, and CCR2
Reelin signaling pathway R-HSA-8866376	.005488	SH3KBP1
Platelet degranulation R-HSA-114608	.008248	PROS1 and VCL
Response to elevated platelet cytosolic Ca2 + R-HSA-76005	.008895	PROS1 and VCL
POU5F1 (OCT4), SOX2, NANOG repress genes related to differentiation R-HSA-2892245	.009858	TSC22D1
Transport of gamma-carboxylated protein precursors from endoplasmic reticulum to Golgi apparatus R-HSA-159763	.009858	PROS1
Removal of amino terminal propeptides from gamma-carboxylated proteins R-HSA-159782	.010948	PROS1
MASTL facilitates mitotic progression R-HSA-2465910	.010948	ENSA
Gamma-carboxylation of protein precursors R-HSA-159740	.010948	PROS1
Gamma-carboxylation, transport, and amino-terminal cleavage of proteins R-HSA-159854	.012036	PROS1

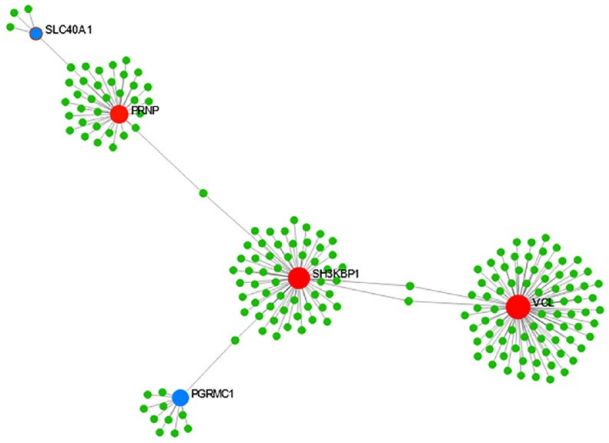

Figure 4. PPI network includes frequently DEGs plus additional STRING database genes. This network contains 187 nodes (5 hub nodes from shared DEGs) and 187 edges.
DEGs indicates differentially expressed genes; PPI, protein-protein interaction.

Discussion

In this work, publicly accessible RNA-seq data of gonorrhea (GSE110106), chlamydia (GSE180238), and prostate cancer (GSE38241) patients were analyzed to determine potential common biomarkers and molecular targets in all 3 diseases. Primarily, those 3 data sets were examined statistically to categorize DEGs, and then, the DEGs of these data sets were taken into account for further investigation. Gene enrichment analysis found that the identified 22 DEGs were mostly responsible for regulation of amyloid precursor protein catabolic process, regulation of amyloid-beta formation, positive regulation of lymphocyte activation, amyloid-beta binding, endopeptidase inhibitor activity, protease binding, nicotinate and nicotinamide metabolism, glycosaminoglycan biosynthesis, regulation of lipolysis in adipocytes, mineral absorption, and so on (Tables 1 and 2). These DEGs were then investigated using the PPI network, which identified 4 hub proteins: VCL, SH3KBP1, PRNP, and PGRMC1 (Figure 4 and Table 3).

Based on information from gene cards database,[26] protein encoded by VCL gene acts as an interacting protein which is involved in anchoring F acting to the membrane. Defect in this gene could lead to dilated cardiomyopathy,[27,28] a condition which leads to congestive heart failure and arrhythmias by ventricular dilation and impaired systolic action. It is also involved in obstructive hypertrophic cardiomyopathy.[29] SH3KBP1 gene encodes an adapter protein which facilitates PPI and is also engaged in several cellular processes including cytoskeleton rearrangement, apoptosis, and cellular adhesion, and disorder associated with this gene includes immunodeficiency.[30] Mutation in PRNP gene is associated with Creutzfeldt-Jakob disease.[31,32] PGRMC1 gene encodes a membrane-associated progesterone steroid receptor which is predominantly expressed in liver and kidney. Disease associated with PGRMC1 gene includes pediatric cataract and breast cancer.[33,34]

As TFs and miRNAs control gene expression at the transcriptional and posttranscriptional levels, alteration in these biomolecules provides fundamental evidence for gene expression dysregulation. So in this work, we investigated common DEG-TF and DEG-miRNA interactions (Tables 3 and 4). Four transcription factors such as POU2F2, POU2F1, GATA6, and HIVEP1 are further examined for determining their association with disease. In accordance with the data from the gene cards database,[26] diseases associated with POU2F2 include B-cell lymphoma and classic Hodgkin lymphoma.[35,36] Diseases associated with POU2F1 include herpes simplex and inflammatory bowel disease[37,38] while diseases associated with GATA6 gene include congenital diaphragmatic hernia, inherited diabetes mellitus, and pancreatic agenesis.[39-41] Mutation in this GATA6 gene also causes human cardiac outflow tract defects[42] and disorders associated with HIVEP1 gene includes immune deficiency diseases.[43]

Among miRNAs (hsa-miR-1224-3p, hsa-miR-485-3p, hsa-miR-125a-5p, hsa-miR-7-5p, hsa-miR-5572, hsa-miR-4717-5p, hsa-miR-153-3p, hsa-miR-506-5p, hsa-miR-4518, and hsa-miR-1266-5p), hsa-miR-7-5p may have role in prostate cancer.[44] According to reports, miR-7 is a tumor suppressor that can stop prostate cancer cells from being stem cells and prevents carcinogenesis by inhibiting KLF4.[45] miR-7 also has been proposed as a potential biomarker in prognosis of prostate cancer.[46] Besides this miR-153 also plays a crucial role in cancer,[47] miR-153 has been proposed as a circulating biomarker for prostate cancer diagnosis.[48] In human prostate cancer, upregulation of miR-153 encourages cell proliferation through downregulation of the PTEN (phosphate and tensin homolog) tumor suppressor gene.[49] hsa-miR-1266-5p may be promising candidates for further research in prostate cancer treatment via the anti-apoptotic pathway.[50]

For identification of drugs, we analyzed protein-drug interaction using the Network Analyst tool which revealed 3 possible therapeutic compounds, namely INCB3284, CCX915, and MLN-1202 (Figure 5). Several reports have been found that CCR2 protein has involvement with prostate cancer.[24,25] According to chEMBL, INCB3284 is an antagonist for CCR2 and currently used to treat inflammatory disorders. It is a selective, potent, and orally bioavailable hCCR2 antagonist.[51] It blocks the stimulation of pro-inflammatory mediators. According to Adis-Insight,[52] CCX915 is a also CCR2 antagonist which is orally available and currently used to treat inflammatory-mediated autoimmune disorders. MLN-1202 is a humanized monoclonal antibody which is designed to treat diabetic nephropathy. It is a G-protein coupled receptor-blocking monoclonal antibody which also has been proposed as a therapeutic agent to treat cancers.[53] These 3 compounds are already in use to treat diseases and have good absorption, distribution, metabolism, and excretion values indicating

Table 3. List of biomarker representatives (proteins and transcription factors) with their biological functions.

BIOMARKER REPRESENTATIVES	FULL FORM	ROLE OF BIOMARKERS
Hub proteins		
VCL	Vinculin	VCL, a cytoskeletal protein, associated with cell-cell and cell-matrix junctions involved in anchoring F-actin to the membrane. Diseases associated with VCL gene include congestive heart failure and cardiomyopathy.
SH3KBP1	SH3 domain containing kinase-binding protein 1	The encoded protein by SH3KBP1 gene promotes protein-protein interactions and has been linked to a variety of cellular processes such as apoptosis, cytoskeletal reorganization, cell adhesion, and the control of clathrin-dependent endocytosis. Diseases associated with SH3KBP1 gene include adrenal cortical adenocarcinoma and immunodeficiency (an X-linked recessive primary immunologic disorder).
PRNP	Prion protein	This gene encodes a membrane glycosylphosphatidylinositol-anchored glycoprotein that aggregates into rod-like structure. Diseases associated with PRNP gene include Creutzfeldt-Jakob disease and Huntington disease-like 1.
PGRMC1	Progesterone receptor membrane component 1	A putative membrane-associated progesterone steroid receptor encodes by this gene. Diseases associated with PGRMC1 gene include premature menopause and cataract.
Transcription factor; $P < .05$		
POU2F2	POU class 2 homeobox 2	A homeobox-containing transcription factor of the POU domain family is encoded by this gene. Diseases associated with POU2F2 gene include B-cell lymphoma and prion disease.
POU2F1	POU class 2 homeobox 1	Diseases associated with POU2F1 gene include herpes simplex and inflammatory bowel disease.
GATA6	GATA-binding protein 6	This gene belongs to a small family of zinc finger transcription factors that regulate cellular differentiation and organogenesis during vertebrate development. Diseases associated with GATA6 include atrioventricular septal defect and congenital anomalies.
HIVEP1	HIVEP zinc finger 1	Isoforms 2 and 3 of this gene may be involved in apoptosis. Diseases associated with HIVEP1 include attention-deficit hyperactivity disorder and Brugada syndrome.

Table 4. Top 10 miRNAs that interact with DEGs generated from miRTarBase2017 database organized by *P* value.

MIRNA NAME	*P*	TARGET GENE	MINIMUM FREE ENERGY OF HYBRIDIZATION (KCAL/MOL)
hsa-miR-1224-3p	.002429	NNT	−37.3
	.002638	KMT2B	−39.4
	.002646	ENSA	−35.2
hsa-miR-485-3p	.002775	TMED10	−25.4
	.002930	SLC40A1	−30.7
hsa-miR-125a-5p	.003236	SLFN11	−29.3
	.003557	NNT	−30.8
	.004152	PTGS1	−30.5
hsa-miR-7-5p	.004330	MARCKSL1	−22.6
	.002429	TMED10	−23.5
	.002638	SLFN11	−22.6
		ENSA	−21.6
hsa-miR-5572	.002646	MARCKSL1	−34.6
	.002775	TMED10	−30.8
hsa-miR-4717-5p	.002930	SH3BGRL	−18.4
	.003236	SLFN11	−28.3
hsa-miR-153-3p	.003557	FAM210B	−20.2
	.004152	KMT2B	−25.3
hsa-miR-506-5p	.004330	PRNP	−23.3
		SLFN11	−24.3
hsa-miR-4518	.002429	MARCKSL1	−28.8
	.002638	NNT	−28.7
hsa-miR-1266-5p	.002646	MARCKSL1	−30.4
		NNT	−29.4

Abbreviations: DEGs, differentially expressed genes; miRNAs, microRNA.

Micro RNA's interactions with target genes were validated using RNAhybrid tool. A negative free energy change indicates spontaneous binding of miRNA with target genes.

promising candidates as drug against the gonorrhea, chlamydia, and prostate cancer.

Pathways and other network analysis could provide valuable understanding toward the development of diagnostic and therapeutic approaches. The found biomarkers and therapeutic potential molecules could be investigated for potential pharmacological targets and activity in the fight against patients with gonorrhea, chlamydia, and prostate cancer.

Conclusions

A large number of people are now suffering from different STDs such as gonorrhea and chlamydia all over the world, and previous history of STDs may influence the development of prostate cancer. In this study, we studied RNA-seq gene expression profiles using computational biology methods to find potential biomarkers that could help us in understanding the patho-biological mechanisms of gonorrhea, chlamydia, and prostate cancer. At the transcriptional and posttranscriptional levels, the identified biomolecules may serve as system biomarkers. Additional research may be necessary to confirm the effectiveness and safety of the compounds found in protein-drug interaction networks as potential treatments for patients with gonorrhea, chlamydia, and prostate cancer.

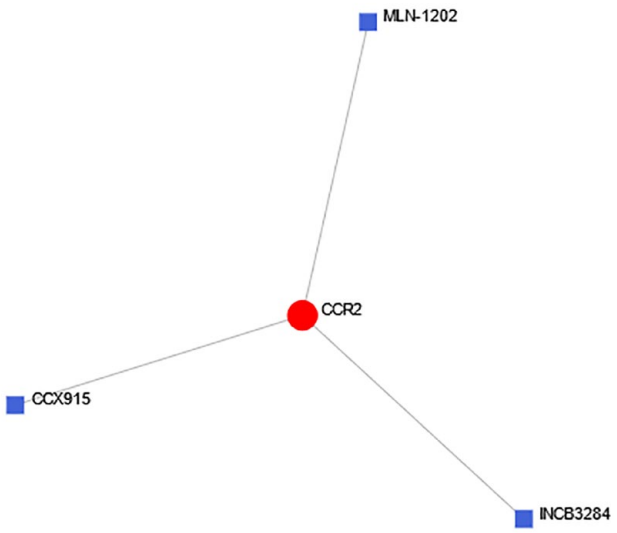

Figure 5. The protein-drug interaction network between hub-protein CCR2 and suggested therapeutics derived from the Network Analyst tool, where the area of each node indicates the degree of interaction. CCR2 indicates C-C chemokine receptor type 2.

Table 5. Interactivity of the 3 well-known drug compounds of drug bank with the common up-regulated gene CCR2 obtained with the help of Network Analyst tool.

ID	LABEL	DEGREE	BETWEENNESS
729230	CCR2	3	3
DB05130	INCB3284	1	0
DB05159	CCX915	1	0
DB05486	MLN-1202/ Plozalizumab	1	0

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Author Contributions

MKI conceived and designed the experiments, made critical revisions, and approved the final version. AAN and MKI analyzed the data and wrote the first draft of the manuscript. MKI, AAN, TF, MMH, and MSKS reviewed the analysis and contributed to the preparation of the manuscript. All authors reviewed and approved the final manuscript.

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