Potential Common Key Genes Associated with Chronic Periodontitis and Low Birth Weight: A Case Control Study Using Bioinformatics Analysis of Pooled mRNA Expression Datasets

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Abstract

Background: Chronic periodontitis (CP) is a multifactorial disease associated with many systemic diseases. However, the precise association between CP and low birth weight (LBW) remains unclear. Therefore, this study aimed to elucidate common differentially expressed genes (DEGs), biomarker candidates, and upstream regulators related to key genes between CP and LBW.

Methods: We investigated molecular relations and biomarker candidates using pooled microarray datasets of CP (GSE12484) and LBW (GSE29807) in the Gene Expression Omnibus (GEO). Datasets were analyzed for common DEGs using GEO2R, an R-based web application for GEO data analysis. Common DEGs, biomarker candidates, and upstream regulators in DEGs between CP and LBW were analyzed using the Database for Annotation Visualization and Integrated Discovery (DAVID), Search Tool for the Retrieval of Interacting Genes (STRING), and QIAGEN's Ingenuity Pathway Analysis (IPA).

Results: Three significantly upregulated and 20 significantly downregulated common DEGs between CP and LBW were identified. Some biological processes and pathways of these downregulated genes were associated with the cell cycle. Biomarker candidates among common DEGs were proline-rich coiled-coil 2A (PPRC2A), topoisomerase (DNA) II alpha (TOP2A), neural cell adhesion molecule 1 (NCAM1), and calcium channel, voltage-dependent, alpha 2/delta subunit 3 (CACNA2D3). Many upstream regulators of these biomarker candidates were factors associated with inflammation, immunity, the cell cycle, and growth development, and were hormones related to pregnancy.

Conclusions: The results of this study suggest that PPRC2A, TOP2A, NCAM1, and CACNA2D3 are common biomedical key genes between CP and LBW. The expression states of these genes, which are related to inflammation, hormones, the cell cycle, and growth development, were common in both CP and LBW in blood. To the best of our knowledge, the relations of PPRC2A, TOP2A, and CACNA2D3 to CP and LBW are reported for the first time. Thus, in the bloodstream, inflammatory-related upstream regulators of these key genes may control gene expression associated with fetal growth, and conversely, changes in female hormones due to pregnancy may affect the progress of CP.

Background

Chronic periodontitis (CP) is characterized by chronic destructive inflammation in periodontal tissues, such as gingiva, cementum, periodontal ligament, and alveolar bone. CP is an inflammatory disease caused by multiple factors, such as immunological response, hormone-regulated gestation, oral bacterial infections, genetic factors, environmental factors, and systematic diseases. Recently, epidemiological studies have reported that CP is related to systemic diseases such as diabetes, rheumatism, cardiovascular disease, chronic kidney disease, premature birth, and low birth weight (LBW; LBW infants are those weighing < 2,500 g at birth). The mechanisms of CP development and progression are complex and have not been completely elucidated. In periodontology, it is important to carry out research on the associations between CP and these systemic diseases to elucidate the mechanisms of CP and identify potential targets for clinical treatment.

Offenbacher et al. first reported the association between CP and LBW in 1996 [1]. Subsequently, many studies were carried out on the association between CP and LBW. Although some epidemiological meta-analyses have reported associations between CP and LBW, the underlying molecular mechanisms remain unclear. The pathogenic bacterium of CP and chemical mediators produced by inflammation in gingiva were considered to penetrate into weak capillary blood vessels, and then to cord blood through the placenta [2–4]. Some researchers have reported that CP influences the development of LBW [5–22], whereas others have denied such an association [23–27]. Therefore, no consensus has been reached on the association between CP and LBW [28–31], and more studies are needed to address this issue. In this study, we focused on the molecular biological interaction between CP and LBW.

We devised a study to investigate the relation between CP and LBW using gene expression profiling in blood. Gene expression profiling is a powerful tool that can elucidate the mechanisms and relations between multifactorial diseases, such as CP and

LBW. Analyzing pooled gene expression datasets with CP or LBW, even under different experimental conditions with different subjects, is useful for investigating common genetic factors.

Data sharing and the integration of pooled omics data to investigate the mechanisms of and relations with multifactorial diseases have received increasing attention as screening tools. The use of pooled microarray gene expression datasets is an effective method for reducing high-throughput hybridization costs and compensating for insufficient amounts of mRNA sampling [32–34]. Thus, the National Center for Biotechnology Information developed the Gene Expression Omnibus (GEO) to promote the pooling and sharing of publicly available transcriptomic data and facilitate biomedical research [35–49].

This study aimed to investigate the common genetic factors and relations between CP and LBW through a bioinformatics analysis of differentially expressed genes (DEGs) using pooled microarray datasets from the GEO database.

Methods

In this study, we used pooled microarray gene expression datasets from the public GEO database to investigate the common molecular factors of CP and LBW as screening tools.

Selection Of Microarray Datasets From The Geo Database

We selected datasets based on the experiential conditions of CP (GSE12484) and LBW (GSE29807) in the National Center for Biotechnology Information (NCBI) GEO database (<http://www.ncbi.nlm.nih.gov/geo/>). These datasets, which include more than two healthy controls/two diseased patients each, were selected based on the typical clinical condition. Samples from peripheral or umbilical cord blood were used, along with a microarray platform (GPL96: Affymetrix GeneChip™ Human Genome U133A Array [HG-U133A] or GPL570: Affymetrix GeneChip™ Human Genome U133 Plus 2.0 Array [HG-U133 Plus 2]) (Table 1).

Table 1
Summary of studies of CP and LBW

Disease	Chronic periodontitis	Low birth weight
GEO dataset ID	GSE12484	GSE29807
Platform	GPL96: Affymetrix GeneChip™ Human Genome U133A Array (HG-U133A)	GPL570: Affymetrix GeneChip™ Human Genome U133 Plus 2.0 Array (HG-U133 Plus 2)
Sample	Peripheral blood	Umbilical cord blood
Number of healthy controls vs. persons with disease	2 vs. 2	4 vs. 8
Diabetes	No	No
Smoking	No	No
Other	No systemic antibiotics or anti-inflammatory drugs for 6 months	No systemic antibiotics or anti-inflammatory drugs for 6 months
PubMed ID	18832737	No

Identification Of Common Up/downregulated Differentially Expressed Genes (degS)

The CP or LBW DEGs between patients and normal healthy controls were identified using GEO2R, an R-based web application for analyzing GEO data. A cutoff value of $p < 0.05$ and a $|\log \text{Fold change (Fc)}| > 1$ were used. Common up/downregulated

DEGs between CP and LBW were then extracted.

Functional And Pathway Enrichment Analysis Of Common Degr

The results of the functional enrichment analysis of common up- or downregulated DEGs based on the BP of GO, and pathway enrichment analyzed based on the KEGG and Reactome pathway using DAVID are shown in Tables 4–6.

Table 4
Biological processes (BP) of downregulated DEGs (FDR < 0.05)

GO term ID	Term description	Observed gene count	Background gene count	FDR	Matching proteins in network
GO:0010639	Negative regulation of organelle organization	5	333	0.0124	ADD2, NEK2, SLC35F6, TOP2A, TTK
GO:0022402	Cell cycle process	7	890	0.0124	CCNB2, CDKN1C, CENPA, CEP76, NEK2, TOP2A, TTK
GO:1903047	Mitotic cell cycle process	6	564	0.0124	CCNB2, CENPA, CEP76, NEK2, TOP2A, TTK
GO:1902850	Microtubule cytoskeleton organization involved in mitosis	3	94	0.0194	CENPA, NEK2, TTK
GO:0071459	Protein localization to chromosome, centromeric region	2	18	0.0222	CENPA, TTK
GO:0000086	G2/M transition of mitotic cell cycle	3	123	0.0262	CCNB2, CEP76, NEK2
GO:0006779	Porphyrin-containing compound biosynthetic process	2	23	0.0262	ABCB6, CPOX
GO:0007143	Female meiotic nuclear division	2	28	0.0262	TOP2A, TTK
GO:0007346	Regulation of mitotic cell cycle	5	608	0.0262	CDKN1C, CEP76, NEK2, TOP2A, TTK
GO:0051304	Chromosome separation	2	25	0.0262	TOP2A, TTK
GO:0006778	Porphyrin-containing compound metabolic process	2	36	0.0327	ABCB6, CPOX
GO:0051726	Regulation of cell cycle	6	1129	0.0327	CCNB2, CDKN1C, CEP76, NEK2, TOP2A, TTK
GO:0033043	Regulation of organelle organization	6	1155	0.0344	ADD2, CEP76, NEK2, SLC35F6, TOP2A, TTK
GO:0015701	Bicarbonate transport	2	44	0.0403	CA1, RHAG
GO:0098813	Nuclear chromosome segregation	3	196	0.0403	NEK2, TOP2A, TTK
GO:0051301	Cell division	4	483	0.0486	CCNB2, CENPA, NEK2, TOP2A
GO:0051321	Meiotic cell cycle	3	214	0.0486	NEK2, TOP2A, TTK
FDR: false discovery rate					

Table 5
KEGG pathway of downregulated DEGs (FDR < 0.05)

KEGG pathway ID	Term description	Observed gene count	Background gene count	FDR	Matching proteins in network
hsa04110	Cell cycle	3	123	0.0077	CCNB2, CDKN1C, TTK

Table 6
Common reactome pathway of downregulated DEGs (FDR < 0.05)

#term ID	Term description	Observed gene count	Background gene count	FDR	Matching proteins in network
HSA-69278	Cell cycle, mitotic	6	483	0.00074	CCNB2, CDKN1C, CENPA, CEP76, NEK2, TOP2A
HSA-1247673	Erythrocytes take up oxygen and release carbon dioxide	2	8	0.0015	CA1, RHAG
HSA-68877	Mitotic prometaphase	4	190	0.0015	CCNB2, CENPA, CEP76, NEK2
HSA-1237044	Erythrocytes take up carbon dioxide and release oxygen	2	12	0.002	CA1, RHAG
HSA-2565942	Regulation of PLK1 Activity at G2/M transition	3	85	0.002	CCNB2, CEP76, NEK2
HSA-380259	Loss of Nlp from mitotic centrosomes	2	68	0.0229	CEP76, NEK2
HSA-8854518	AURKA activation by TPX2	2	71	0.0229	CEP76, NEK2
HSA-380270	Recruitment of mitotic centrosome proteins and complexes	2	79	0.024	CEP76, NEK2
HSA-380320	Recruitment of NuMA to mitotic centrosomes	2	91	0.0276	CEP76, NEK2
HSA-5620912	Anchoring of the basal body to the plasma membrane	2	96	0.0287	CEP76, NEK2
HSA-382551	Transport of small molecules	4	706	0.0319	ABCB6, ADD2, CA1, RHAG
HSA-2500257	Resolution of sister chromatid cohesion	2	118	0.0381	CCNB2, CENPA
FDR: false discovery rate					

Common upregulated DEGs were not significantly enriched in BP, KEGG, or the Reactome pathway. Common downregulated DEGs were significantly enriched in BP, KEGG, and the Reactome pathway (Tables 4–6). Predominantly, common downregulated DEGs were related to the cell cycle and metabolic processes in BP, and to the cell cycle, cell composition, erythrocyte function, and transport pathways for small molecules in KEGG and the Reactome pathway.

Constructed Protein–protein Interaction (ppi) Networks Of Common Degr

Constructed PPI networks of common up- and downregulated genes were identified using the Search Tool for the Retrieval of Interacting Genes (STRING) (<https://string-db.org/cgi/about.pl>).

Elucidation Of Common Molecular Biomarker Candidates

Common molecular biomarker candidates between CP and LBW were identified using IPA software (Table 7). Biomarker candidates are used to identify disease states such as diagnosis, efficacy, disease progression, and prognosis. Proline-rich coiled-coil 2A (PRRC2A) was a significant common upregulated DEG, whereas calcium channel, voltage-dependent, alpha 2/delta subunit 3 (CACNA2D3), neural cell adhesion molecule 1 (NCAM1), and TOP2A were significant common downregulated DEGs.

Table 7
Common molecular biomarker candidates for diagnosis, prognosis, and other processes

Gene symbol	Entrez gene name	Up- or downregulated DEG	Biomarker application(s)
IFIT2	Interferon-induced protein with tetratricopeptide repeats 2		Diagnosis
TOP2A	DNA topoisomerase II alpha	Downregulated DEG	Diagnosis, efficacy, prognosis, response to therapy
ATF6	Activating transcription factor 6		Efficacy
TNFSF10	TNF superfamily member 10		Efficacy
NCAM1	Neural cell adhesion molecule 1	Downregulated DEG	Efficacy, prognosis, unspecified Application
CACNA2D3	Calcium channel, voltage-dependent, alpha 2/delta subunit 3	Downregulated DEG	Prognosis
GART	Phosphoribosylglycinamide formyltransferase, phosphoribosylglycinamide synthetase, phosphoribosylaminoimidazole synthetase		Prognosis, safety
PRRC2A	Proline-rich coiled-coil 2A	Upregulated DEG	Safety
PVT1	Pvt1 oncogene		Safety

Analysis Of Upstream Regulators Of Dominant Common Degr

Upstream regulators of common DEGs were analyzed using comparison analysis in IPA software. DEGs were uploaded into the IPA software, and genetic networks were analyzed in the Ingenuity Knowledge Base. We also analyzed the functional annotations of upstream regulators.

Results

We investigated common DEGs, BP and pathway analyses, biomarker candidates, and upstream regulators between CP and LBW through gene expression profiling of the GEO datasets.

Identification Of Common Degr

Common DEGs were identified from among respective DEGs investigated using GEO2R, and genes involved in the pathogenesis of CP and LBW were elucidated. Three significantly upregulated and 20 significantly downregulated common DEGs between CP and LBW were identified, as shown in Tables 2 and 3 ($p < 0.05$, $|\log Fc| > 1$). Venn diagrams representing the overlap of DEGs between CP (GSE12484) and LBW (GSE29807) are shown in Fig. 1.

Table 2
Significant common upregulated DEGs ($p < 0.05$, $\log FC > 1$)

Gene symbol	Gene title	Affy ID	Chronic periodontitis		Low birth weight	
			logFC	p-value	logFC	p-value
PRRC2A	Proline-rich coiled-coil 2A	208132_x_at	1.447139	0.00284602	1.17	0.04361717
RPS2	Ribosomal protein S2	217466_x_at	1.6966286	0.04138839	2.38	0.0281293
SNORA64	Small nucleolar RNA, H/ACA box 64	217466_x_at	1.6966286	0.04138839	2.38	0.0281293

Table 3
Significant common downregulated DEGs ($p < 0.05$, $\log FC < -1$)

Gene symbol	Gene title	Affy ID	Chronic periodontitis		Low birth weight	
			logFC	p-value	logFC	p-value
ABCB6	ATP binding cassette subfamily B member 6	203192_at	-1.0569106	0.0431129	-1.31	0.00294847
ADD2	Adducin 2	205268_s_at	-1.1046457	0.00777222	-1.94	0.00917617
ALDH6A1	Aldehyde dehydrogenase 6 family, member A1	221590_s_at	-2.2681268	0.00798702	-1.26	0.03233938
CA1	Carbonic anhydrase 1	205950_s_at	-1.5793517	0.02485654	-1.63	0.04056291
CACNA2D3	Calcium channel, voltage-dependent, alpha 2/delta subunit 3	219714_s_at	-1.888585	0.00172782	-1.18	0.00337368
CCNB2	Cyclin B2	202705_at	-1.1595288	0.04481641	-1.43	0.00690167
CDKN1C	Cyclin-dependent kinase inhibitor 1C	213348_at	-1.3070001	0.00646845	-1.19	0.04085004
CENPA	Centromere protein A	204962_s_at	-2.1527643	0.04419247	-1.2	0.01221934
CEP76	Centrosomal protein 76	52285_f_at	-1.2023041	0.00843916	-1.18	0.00192199
CPOX	Coproporphyrinogen oxidase	204172_at	-1.0987262	0.02304834	-2.47	0.00275945
ENPP4	Ectonucleotide pyrophosphatase/phosphodiesterase 4	204161_s_at	-1.8863298	0.01127334	-1.8	0.00230219
NCAM1	Neural cell adhesion molecule 1	209968_s_at	-2.1606266	0.00608607	-1.39	0.00440039
NEK2	NIMA-related kinase 2	204641_at	-2.4662128	0.01606756	-1.73	0.00514136
RHAG	Rh-associated glycoprotein	206145_at	-2.4662496	0.00254827	-2.67	0.01323244
RHOBTB1	Rho-related BTB domain-containing 1	212651_at	-1.21743	0.01193424	-1.05	0.0108552
SDAD1	SDA1 domain-containing 1	218607_s_at	-1.3541992	0.00680884	-1.68	0.00547458
SLC35F6	Solute carrier family 35 member F6	204962_s_at	-2.1527643	0.04419247	-1.2	0.01221934
TOP2A	Topoisomerase (DNA) II alpha	201292_at	-1.7960199	0.00205856	-1.89	0.00030621
TTK	TTK protein kinase	204822_at	-1.3045337	0.00430803	-1.03	0.00196834
ZNF184	Zinc finger protein 184	213452_at	-1.0276901	0.02369172	-1.16	0.03112214

Construction Of Ppi Networks Of Common Degs

PPI networks of common up- and downregulated DEGs are shown in Fig. 2. Upregulated DEGs had no relation to each other, whereas downregulated DEGs constructed a few low-degree PPI networks, such as ATP-binding cassette subfamily B member 6 (ABCB6), coproporphyrinogen oxidase (CPOX), cyclin B2 (CCNB2), cyclin-dependent kinase inhibitor 1C (CDKN1C), centromere protein A (CENPA), centrosomal protein 76 (CEP76), NIMA-related kinase 2 (NEK2), topoisomerase (DNA) II alpha (TOP2A), and TTK protein kinase (TTK).

Upstream Regulators Of Dominant Common Biomarker Candidates

Upstream regulators of common DEGs between CP and LBW, such as PRRC2A, TOP2A, NCAM1, and CACNA2D3, were revealed using IPA software.

In this study, upstream regulators of PRRC2A and CACNA2D3 showed inhibitory reactions, while those of TOA2A and NCAM1 showed inhibitory or active reactions (Table 8).

Discussion

CP is a multifactorial disease, and relations between CP and systemic diseases have gathered increasing attention. The association between CP and LBW was first reported by Offenbacher et al. [1]. Many epidemiological studies have reported that pregnant women with CP are several times more likely than women without CP to have a preterm LBW infant [5–22]. Additionally, some studies have found that treatment for periodontitis is effective for preventing LBW [45–48]. Those studies reported that CP was an important risk factor for LBW. Specifically, hormonal alterations during pregnancy can cause bacterial infections to progress into periodontitis [2–4, 49]. Chronic inflammation with CP induces the production of some proinflammatory cytokines [50–52]. Moreover, molecular studies have reported increased placental expression of interleukin-1 beta (IL-1 beta), cyclooxygenase-2 (COX-2), vascular endothelial growth factor receptor (VEGFR1), and heat shock protein (HSP70) in patients with CP [53]. These inflammatory mediators cause uterine contraction and vasoconstriction, which lead to LBW [54].

However, conversely, some studies have reported finding no association between CP and preterm LBW, and that treatment for periodontitis had no effect on the prevention of LBW [55–66]. Furthermore, as LBW is related to numerous risk factors, such as the mother's age, onset of prenatal care, systemic diseases, previous LBW infants, complications during pregnancy, and term of delivery, CP may not be an important risk factor for LBW [23–27, 67]. Thus, the precise association between CP and LBW remains unclear.

In this study, we focused on common genetic factors and molecular interactions between CP and LBW by performing gene expression analyses with pooled datasets from the GEO database.

Microarray analysis is a powerful tool to identify new candidate genes involved in the gene expression profiling of multifactorial diseases. Gene expression profiling involves the comprehensive study of gene expression levels; these can be used to diagnose a disease or predict treatment effects. The NCBI GEO database is the largest public repository for high-throughput biological assays generated by the research community [35–44].

In addition, data sharing and the integration of pooled omics data for investigations of biomedical mechanisms and multifactorial disease relations have gained increasing attention. Using pooled microarray gene expression datasets from the GEO is a method that reduces high-throughput hybridization costs and compensates for insufficient amounts of mRNA sampling [32–34].

In this study, we analyzed microarray gene expression datasets from the GEO database to elucidate the association between CP and preterm LBW. Although the two datasets contain different experiment conditions, subjects, and diseases, the relation

between common genetic factor and biological interaction candidates and multifactorial diseases such as CP and LBW may be elucidated as screening tools.

Common genetic factors, molecular pathways, genetic interactions, and biomarker candidates between CP and LBW were analyzed using DAVID, STRING, and IPA. DAVID is a web-accessible program that provides a comprehensive set of functional annotation tools for investigators to understand biological meanings behind large lists of given genes [68]. STRING is a database of known and predicted PPIs of multiple proteins [69]. IPA is an application built on a large knowledge database acquired by curators. IPA is a powerful application for the discovery of upstream regulators and biomarker candidates with omics data such as microarray analysis that identifies new biomarkers within the context of biological systems [70, 71].

The aim of this study was to elucidate key genes and biological interactions between CP and LBW using bioinformatics analysis of microarray datasets in the GEO database.

We examined important common factors and their functions related to CP and LBW. The functions of the genes were considered while referring to the information in NCBI GEO database [72].

Our analysis of CP and LBW gene expression profiles identified three significantly upregulated DEGs and 20 significantly downregulated DEGs. The three upregulated DEGs had no significant relation with each other. Among the three upregulated DEGs, PRRC2A can be assumed to be associated with inflammation and immunity as it is localized in the vicinity of the genes for tumor necrosis factors alpha and beta [72]. PRRC2A is associated with rheumatoid arthritis and the age at onset of insulin-dependent diabetes mellitus [72]. Some downregulated DEGs, such as CCNB2, CDKN1C, CENPA, CEP76, NEK2, TOP2A, and TTK, were found to be related to the cell cycle from the functional analysis of the BP and pathway databases. Based on the PPI networks, TOP2A had direct interactions with the downregulated DEGs: CCNB2, TTK, NEK2, and CENPA.

The results of the IPA biomarker analysis showed that interferon-induced protein with tetratricopeptide repeats 2 (IFIT2), TOP2A, activating transcription factor 6 (ATF6), TNF superfamily member 10 (TNFSF10), NCAM1, CACNA2D3, phosphoribosylglycinamide formyltransferase, phosphoribosylglycinamide synthetase, phosphoribosylaminoimidazole synthetase (GART), PRRC2A, and Pvt1 oncogene (PVT1) were common molecular biomarker candidates.

Based on the upstream regulator analysis, catenin beta 1 (CTNNB1) and interleukin-5 (IL-5) were found to be the upstream regulators suppressing PRRC2A, which is one of the upregulated DEGs. CTNNB1 is involved in the bonding of cell adhesion molecules, the homeostasis of living organisms, and intracellular messenger activity [72]. IL-5 is a hematopoietic cytokine that plays an important role in the differentiation, maturation, mobilization, and activation of neutrophils [72].

TOP2A, NCAM1, and CACNA2D3 were identified as common downregulated DEGs, while beta-estradiol, transforming growth factor beta 1 (TGFB1), trichostatin A, and decitabine were identified as common upstream regulators showing inhibitory reactions to TOP2A and NCAM1. Sirolimus was found to be an upstream regulator showing active reactions to TOP2A and NCAM1.

As for CACNA2D3, there is nothing in common upstream regulators with TOP2A and NCAM1. Adenylate cyclase activating polypeptide 1 (ADCYAP1), musculoaponeurotic fibrosarcoma oncogene homolog B (MAFB), achaete-scute homolog 1 (ASCL1), nuclear receptor subfamily 3 group C member 2 (NR3C2), and pancreas transcription factor 1 subunit alpha (PTF1A) were found to be active upstream regulators of CACNA2D3. ADCYAP1 is a transduction material, MAFB is involved in the differentiation of hematopoietic stem cells to monocytes and macrophages, ASCL1 is a transcription factor required when cells differentiate into neurons involved in the nuclear receptor of steroids, such as NR3C2 [72].

The results of this study revealed that PRRC2A, TOP2A, NCAM1, CACNA2D3, CTNNB1, IL5, ASCL1, NR3C2, ADCYAP1, and MAFB are genes commonly associated with CP and LBW, and that upstream regulators such as lipopolysaccharide and pregnancy-associated hormones are dominant regulators commonly associated with CP and LBW. These key genes and regulators are related to not only inflammation and immunity, but also the cell cycle, the bonding of cell adhesion molecules, intercellular messenger activity, the homeostasis of living organisms, and cell differentiation.

Previously reported genes and regulators related to both CP and LBW in the PubMed database are shown in Table 9. In this study, BCL2/adenovirus E1B 19 kDa protein-interacting protein 3-like (BNIP3L) and cyclin dependent kinase inhibitor 1A (CDKN1A) were found to be activated upstream regulators of TOP2A. Beta-estradiol, CD24, erb-b2 receptor tyrosine kinase 2 (ERBB2), estrogen, lipopolysaccharide, peroxisome proliferator-activated receptor alpha (PPARA), TGFB1, and tretinoin are inhibited upstream regulators of TOP2A, while beta-estradiol, TGFB1, and tretinoin are common inhibited upstream regulators of TOP2A and NCAM1, and NCAM1 is a common downregulated DEG and biomarker. Many of the listed genes and regulators are related to cell generation, development, and organization. Some genes and regulators were found to be indirectly related to immunology and inflammation, while some hormones related to pregnancy and fetal growth were found to influence CP and LBW as upstream regulators. The pooled omics data microarray analysis carried out in this study revealed that several genes related to CP and LBW have functions relevant to cell morphology, organ morphology, and skeletal and muscular diseases, in addition to inflammation and immunity.

Table 8
Upstream regulators of dominant common biomarker candidates

Table 8. Upstream regulators of dominant common biomarker candidates		
Target gene (up- or downregulated DEG)	Upstream regulator	Predicted activation state
PRRC2A (upregulated DEGs)	CTNNB1, IL5	Inhibited
TOP2A (downregulated DEG)	dexamethasone, TGFB1, beta-estradiol, estrogen, trichostatin A, diethylstilbestrol, IL6, FGF2, NRG1, OSM, testosterone, MYOD1, RABL6, YAP1, E2F1, P38 MAPK, poly rI:rc-RNA, lipopolysaccharide, BRD4, NR1H3, PPARA, EWSR1, FOXM1, ERBB2, decitabine, CFS2, CD3, tretinoin, LLLGL2, CD24, ELAVL1, NSUN6, trans-hydroxytamoxifen, raloxifene	Inhibited
	LY294002, mir-21, PD98059, TCF3, dexamethasone, sirolimus, CDKN1A, BNIP3L, JQ1, KRAS, curcumin, 26 s Proteasome	Activated
NCAM1 (downregulated DEG)	TGFB1, beta-estradiol, tretinoin, progesterone, CTNNB1, trichostatin A, EGF, MYC, IGF1, bucladesine, WNT3A, NFkB (complex), JUN, BMP7, phytohemagglutinin, BMP4, OTX2, BDNF, CD38, NEUROD1, PAX8, EPHB4, decitabine, SOX4, CUX1, TNF, Ngf, monocrotaline	Inhibited
	MYCN, sirolimus, NOG, miR-182-5p, KRAS, curcumin, mir-210	Activated
CACNA2D3 (downregulated DEG)	ADCYAP1, MAFB, ASCL1, NR3C2, PTF1A	Inhibited

Table 9
Genes and regulators reported to be related to both CP and LBW (PubMed)

Genes and regulators reported in previous studies	Relation in this study
Beta-estradiol	Inhibits upstream regulators of TOP2A and NCAM1
BNIP3L	Activates upstream regulator of TOP2A
Camptothecin	
CD24	Inhibits upstream regulator of TOP2A
CDKN1A	Activates upstream regulator of TOP2A
Cisplatin	
CYP3A4	
Doxorubicin	
ERBB2	Inhibits upstream regulator of TOP2A
ESR1	
Estrogen	Inhibits upstream regulator of TOP2A
FAS	
GNAS	
HFE	
L-dopa	
Lipopolysaccharide	Inhibits upstream regulator of TOP2A
MDM2	
mir-21	Activates upstream regulator of TOP2A
MTHFR	
NCAM1	Common downregulated gene, common biomarker
PPARA	Inhibits upstream regulator of TOP2A
Rb	
Sos	
TGFB1	Inhibits upstream regulators of TOP2A and NCAM1
TP53	
Tretinoin	Inhibits upstream regulators of TOP2A and NCAM1
YY1	

Conclusions

Investigations on the relation between phenotype and gene expression levels are important to elucidate biological-related factors in various diseases. Using the integrated analysis of omics data, such as those from microarray mRNA expression datasets, enables DEGs, genetic networks, common biomarker candidates, and upstream regulators to be identified, which is important not only for prognosis, diagnosis, and medical treatment, but also for the elucidation of the molecular mechanisms of multifactorial diseases as screening tools.

The results of this study suggest that PRRC2A, TOP2A, NCAM1, and CACNA2D3 may be important common key genes related to CP and LBW. To our knowledge, this is the first study to report the relations between PRRC2A, TOP2A, and CACNA2D3 and CP and LBW. These key genes are related to the cell cycle, cell composition, erythrocyte function, and transport pathways for small molecules. Among the upstream regulators of key genes, hormones such as beta-estradiol, estrogen, and progesterone had indirect effects on inflammation and immunity, whereas others had direct effects.

Therefore, inflammation-related factors caused by CP may influence gene expression associated with fetal growth. Conversely, female hormones related to pregnancy may affect the progress and development of CP. These predicted molecular key genes obtained from bioinformatics analysis should be further validated in future experimental research.

List Of Abbreviations

ABCB6: ATP-binding cassette subfamily B member 6; ADCYAP1: Adenylate cyclase-activating polypeptide 1; ASCL1: Achaete-scute homolog 1; ATF6: Activating transcription factor 6; BP: Biological process; CACNA2D3: Calcium channel, voltage-dependent, alpha 2/delta subunit 3; CCNB2: Cyclin B2; CDKN1C: Cyclin-dependent kinase inhibitor 1C; CENPA: Centromere protein A; CEP76: Centrosomal protein 76; COX-2: Cyclooxygenase-2; CP: Chronic periodontitis; CPOX: Coproporphyrinogen oxidase; CTNNB1: Catenin beta 1; DAVID: Database for Annotation Visualization and Integrated Discovery; DEGs: Differentially expressed genes; ERBB2: erb-b2 receptor tyrosine kinase 2; Fc: Fold change; GART: Phosphoribosylglycinamide formyltransferase, phosphoribosylglycinamide synthetase, phosphoribosylaminoimidazole synthetase; GEO: Gene Expression Omnibus; GO: Gene ontology; HSP70: Heat shock protein; IFIT2: Interferon-induced protein with tetratricopeptide repeats 2; IL-1 beta: Interleukin-1 beta; IL-5: Interleukin-5; IPA: Ingenuity Pathway Analysis; KEGG: Kyoto Encyclopedia of Genes and Genomes; LBW: Low birth weight; MAFB: Musculoaponeurotic fibrosarcoma oncogene homolog B; NCAM1: Neural cell adhesion molecule 1; NCBI: the National Center for Biotechnology Information; NEK2: NIMA-related kinase 2; NR3C2: Nuclear receptor subfamily 3 group C member 2; PPARA: peroxisome proliferator-activated receptor alpha; PPI: Protein–protein interaction; PRRC2A: Proline-rich coiled-coil 2A; PTF1A: pancreas transcription factor 1 subunit alpha; PVT1: Pvt1 oncogene; STRING: Search Tool for the Retrieval of Interacting Genes; TGFB1: Transforming growth factor beta 1; TOP2A: Topoisomerase (DNA) II alpha; TNFSF10: Tumor necrosis factor superfamily member 10; TTK: TTK protein kinase; VEGFR1: Vascular endothelial growth factor receptor 1

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and analyzed in this study are available in the GEO datasets repository at <https://www.ncbi.nlm.nih.gov/gds>.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

AS conceived this study, participated in the design, and performed the statistical analysis. TH, AN, and EK participated in the design and helped draft the manuscript. YN helped draft the manuscript. All authors read and approved the final manuscript.

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Figures

a

b

Figure 1

Venn diagrams representing the overlaps between two GEO datasets. (a) the identification of upregulated DEGs. (b) The identification of downregulated DEGs.

Figure 2

Up and down regulated genes and biomarkers RPA2 and PRRC2A are up regulated genes, ABCB6, ADD2, CA1, CACNA2D3, CCNB2, CDKN1C, CENPA, CEP76, CPOX, ENPP4, NCAM1, NEK2, RHAG, RHOBTB1, SDAD1, SLC35F6, TOP2, TTK and ZNF184 are down regulated genes. PRRC2A, CACNA2D3, NCAM1 and TOP2A are biomarker candidates. TOP2 had direct interaction CCNB2, TTK, NEK2, SDAD1 and CENPA which were down regulated genes. There is no protein-protein interaction among the up regulated genes such as RPS2 and PRRC2A. There are, however, some protein-protein interactions among the down regulated genes with low degree.