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Biomarker screening in fetal growth restriction based on multiple RNA-seq studies

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ABSTRACT

Objective: Fetal growth restriction (FGR) is a severe pathological complication associated with compromised fetal development. The early diagnosis and prediction for FGR are still unclear. Sequencing technologies present a huge opportunity to identify novel biomarkers. However, limitation of individual studies (e.g., long lists of dysregulated genes, small sample size and conflicting results) hinders the selection of the best-matched ones.
Study design: A multi-step bioinformatics analysis was performed. We separately reanalyzed data from four public RNA-seq studies, followed by a combined analysis of individual results. The differentially expressed genes (DEGs) were identified based on DESeq2. Then, function enrichment analyses and protein-protein interaction network (PPI) were conducted to screen for hub genes. The results were further verified by using external microarray data.
Results: A total of 65 dysregulated genes (50 down and 15 upregulated) were identified in FGR compared to controls. Function enrichment and PPI analysis revealed ten hub genes closely related to FGR. Validation analysis found four downregulated candidate biomarkers (*CEACAM6*, *SCUBE2*, *DEFA4*, and *MPO*) for FGR.
Conclusions: The use of omics tools to explore mechanism of pregnancies disorders contributes to improvements in obstetric clinical practice.

1. Introduction

FGR, a severe pathological condition that refers to fetuses failing to reach their growth potential, complicates approximately 5–10% of all pregnancies worldwide [1,2]. Placental dysfunction, a disorder characterized by insufficient vascular remodelling of the spiral arteries and suboptimal uterine-placental perfusion, is a major contributing factor to FGR [3,4]. However, the underlying pathology of FGR is still not fully understood. The mystery of the pathology underlying FGR also made early prediction and diagnosis of FGR challenging.

One approach to finding biomarkers is to use omic methods. Gene expression profiling can be used to identify novel prognostic and diagnostic biomarkers [5]. For example, fms-like tyrosine kinase-1(sFLT1), an upregulated gene identified by microarray, has now been applied to clinical screening in developing pre-eclampsia [6]. Several diagnostic biomarkers have been suggested for FGR: pregnancy-associated plasma protein A (PAPP-A), placental growth factor (PlGF), and sFLT1 [7–9]. More recently, maternal serum levels of follistatin-like 3 (FSTL3) protein were found predictive of FGR [10]. However, none are currently clinically recommended consistently worldwide.

Recent studies using gene sequencing techniques expand our knowledge of the placental transcriptional landscape in pregnancy complications [11], including FGR. RNA sequencing (RNA-seq) research, based on next-generation sequencing technology, is flourishing. The deposition of sequencing data in public databases, such as the Sequence Read Archive (SRA) and Gene Expression Omnibus (GEO), facilitates global data sharing. However, results from individual RNA-seq studies differ, leading to different conclusions. In addition, the small number of biological replicates in each separate study limits the credibility and accuracy of the results. For these reasons, this study aimed to identify potential disease-specific biomarkers by combining data from different backgrounds to show a comprehensive transcriptome picture from multiple studies.

In this study, we utilized an established comprehensive workflow to identify of FGR-specific biomarker candidates with reduced impact from laboratory bias [12]. We separately reanalyzed data from four RNA-seq

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studies of FGR, followed by a combined analysis of the individual results to reduce false-positive rate. Besides, we addressed the selection of suitable housekeeping genes for subsequent biomarker expression analyses. Therefore, this study will help to discover novel biomarkers that may provide evidence for predicting and managing FGR.

2. Methods

2.1. RNA-seq data collection and processing

The available databases were searched to select projects including placental transcriptomic data from physiological and FGR-affected pregnancies. For RNA-seq profiles of studies related to fetal growth restriction in four datasets were downloaded from SRA database (https://www.ncbi.nlm.nih.gov/sra): PRJEB30656 – a Polish study on placenta transcriptome of FGR pregnancies which were diagnosed considering both intrauterine vessels flow index and estimated fetal weight [13]; PRJEB37698 – a Polish study on placental sex-biased long non-coding RNAs signature in FGR [14]; PJNA472249 – a Canadian study on placental microRNA regulation in early-onset FGR [15]; PRJNA894845 – a Chinese study on placenta transcriptome in term FGR (Xiaohui Li, Xin He, Yanan Hu, Zhengpeng Li, Yi Chen, 2023, unpublished data). Sequencing data in all studies were performed by Illumina platform.

The raw RNA sequencing data (fastq files) were downloaded using the corresponding accession numbers and assessed for quality control using the fastqc (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). fastp was applied to eliminate adaptors and trim reads with a low phread quality (cutoff < 20) [16]. Reads that passed the quality control metrics were mapped to the human reference genome (GRCh38) with HISAT2 [17]. Samtools software [18] was used to convert BAM to SAM format. Finally, the mapped reads of each sample were assembled by featurecounts [19] with ENSEMBL annotation (GRCh38.107) at both gene and transcript levels.

2.2. Biomarker screening analysis

Gene count expression matrix was examined by the DESeq2 [20]. When the statistical tests satisfied the following thresholds: (1) fold change > 2 or < 0.5; (2) p-value < 0.05, the gene was considered as a differentially expressed within a study. The false-negative rate of identifying differentially expressed genes (DEGs) was further reduced in subsequent comparisons of each individual studies, as previously described [12]. Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways enrichment analyses were carried out with the g:Profiler [21] using the DEGs. To figure out the potential protein interactions, protein-protein interaction (PPI) network of the DEGs was investigated using the STRING database (version 11.5; http://string-db.org/) and STRINGdb R package with default interaction scores threshold (cutoff = 400). The Maximal Clique Centrality (MCC) topological algorithm of CytoHubba program in Cytoscape software was used to identify the key gene modules [22].

2.3. Potential biomarker validation using external transcriptomic dataset

Selected candidate DEGs for FGR were validated by comparison with external data in similar studies. Microarray data (ID: GSE75010) dataset were chosen for further analysis. Pregnancies with maternal hypertension were excluded. Gene expression levels were normalized as RMA when comparing between groups.

2.4. Statistics analysis

R (version 4.1.0, http://www.r-project.org) was used for statistical analysis and visualization. Differences between groups of continuous variables were tested using Wilcoxon test. P-values were considered statistically significant at $p < 0.05$.

3. Results

3.1. Biomarker screening using multiple RNA-seq studies

To identify promising and potential novel biomarker candidates, we reanalyzed data from four publicly available placental RNA-seq studies for FGR, i.e., PRJEB30656 [13], PRJEB37698 [14], PRJNA472249 [15], and PRJNA894845 (Xiaohui Li, 2023, unpublished data). Within each study, we first identified the dysregulated genes using fairly relaxed filtering conditions with DESeq2, followed by a comparative analysis of all studies, as described in [12]. We used two different approaches to identify DEGs by either based on the genome (gene IDs) or the transcriptome (transcript IDs). The number of DEGs varied between the individual studies: 3669, 1079, 12033, and 1388 DEGs in PRJEB30656, PRJEB37698, PRJNA472249 and PRJNA894845, respectively, when mapping on the genome; and 3459, 1181, 13984, and 1640 DEGs in PRJEB30656, PRJEB37698, PRJNA472249 and PRJNA894845, respectively, when mapping on the transcriptome (Table 1). The directions of the regulation for DEGs were shown in Fig. S1.

We next compared DEGs between all four studies to identify common dysregulation genes. For subsequent analyses, we only included DEGs observed in at least three studies (with an unadjusted P value < 0.05) and were consistent with the direction either upregulated or downregulated. We obtained a total of 223 such genes (155 downregulated and 68 upregulated; **175** protein-coding genes) (Fig. 1a; mmc2.xlsx) and 206 transcripts (125 downregulated and 81 upregulated; representing **154** unique protein-coding genes) (Fig. 1b; mmc3.xlsx). Of note, eight genes (four protein-coding genes) and five transcripts (two protein-coding genes) were identified in all four studies. The distribution of DEGs in the four FGR RNA-seq studies without considering the directionality of the regulation was shown in Fig. S2.

We then compared the DEGs obtained at the gene and transcript levels, resulting in 65 common protein-coding genes dysregulated in at least three studies. Fifty genes were downregulated, and 15 were upregulated in at least three studies (Fig. 2). Especially, *S100A12* was downregulated in all four studies at gene level, while *S100A12* and *RHOQ* were downregulated in all four studies at transcript level (Fig. 2). The study PRJNA894845 showed reduced intensities of dysregulation: large numbers of genes are dysregulated, however, with P values greater than 0.05 (shown in grey).

3.2. Functional and pathway enrichment of candidate DEGs

We then carried out the gene set enrichment analysis using the previously identified 65 overlapping candidate DEGs. Here, we identified four biological processes (BP), four cellular components (CC), and eight molecular functions (MF), but no KEGG pathway (Fig. 3; mmc4.xlsx). We observed some items were closely associated with cell signal communication, such as cell-cell signaling, signaling receptor binding, signaling receptor activator activity, receptor ligand activity, hormone activity and glycosaminoglycan binding.

3.3. PPI network analysis of candidate DEGs

According to data from the STRING database, the gene interaction network had 65 edges and 26 nodes (Fig. 4). To identify the significant contributor to this PPI network module, we utilized the CytoHubba program in Cytoscape to find core gene nodes. *MPO, VCAN, S100A12, DEFA4, ADAMTS7, SCUBE2, VTN, CEACAM6, OLFM4* and *STX1A* were the top ten high-degree hub nodes selected by the topological methods. Additionally, functional enrichment analysis revealed that these ten genes were primarily involved in glycosaminoglycan binding (GO:MF: 0005539), defense response to fungus (GO:BP:0050832) and secretory granule (GO:CC: 0030141) (mmc5.xlsx).

On the basis of the DEGs identified, we next aimed to identify possible biomarker candidates for FGR. To further verify the mRNA

Table 1
Differentially expressed genes (DEGs) identified in four publicly available placenta RNA-seq studies for fetal growth restriction (FGR).

Project ID	Control samples	FGR samples	DEGs (gene)	Percentages of all genes	DEGs (transcript)	Percentages of all transcripts
PRJEB30656	5	5	3669	5.9%	3459	1.4%
PRJEB37698	6	6	1079	1.7%	1181	0.5%
PRJNA472249	21	18	12033	19.5%	13984	5.6%
PRJNA894845	6	6	1388	2.2%	1640	0.7%

The numbers of control and FGR samples, as well as the numbers of dysregulated DEGs (P value < 0.05) within each study, are shown.

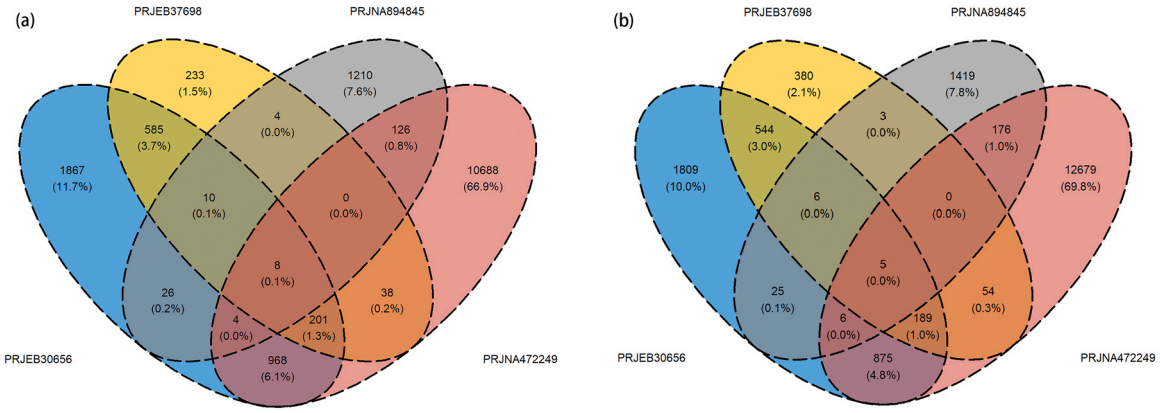

Fig. 1. Distribution of differentially expressed genes (DEGs) in the four RNA-seq studies considering the directionality of the regulation. (a) analyzed at the gene level; (b) analyzed at the transcript level.

expression levels of the hub genes, the data of FGR fetuses were obtained from a public database (ID: GSE75010) (Fig. S3). Expression changes of *CEACAM6*, *MPO*, *DEFA4* and *SCUBE2* were further confirmed using this external transcriptomic dataset (Fig. 5). Therefore, the genes *CEACAM6*, *MPO*, *DEFA4* and *SCUBE2* may be possible pathogenic target of FGR by combining PPI analysis.

3.4. Selection of housekeeping genes using public RNA-seq data

Additional analyses useful in biomarker screening can be achieved using public RNA-seq data. Selecting a suitable housekeeping gene (with a stable expression in the context of FGR) is crucial for subsequent gene expression analyses (e.g., real-time quantitative PCR). We analyzed 20 commonly used housekeeping genes at gene level comparing the four FGR RNA-seq studies mentioned above (Fig. S4; mmc6.xlsx). Most of the genes showed dysregulation ($p < 0.05$) in at least one of the studies. However, *CYB561, CYC1, HBS1L, RPLP0* and *TBP* showed no dysregulation in all four studies within the context of FGR, which we suggest as suitable housekeeping genes.

4. Discussion

Recently, the rapid development of omic tools has presented a huge opportunity to identify novel biomarkers for pregnancy complications. Various methods to screen for potential FGR biomarkers have been reported. These include transcriptomics [10], proteomics [23], metabolomics [24] and epigenetics [25]. However, they might yield hundreds or thousands of identified DEGs at last, generating overly optimistic predictions. Most omic studies assessing FGR also displayed a common limitation: small sample sizes with homogeneous ethnic backgrounds. In the present study, we performed a multi-step bioinformatics analysis of multiple RNA-seq data, following the established workflow [12], to identify hub genes as potential biomarkers of FGR. With the help of transcriptome profiles of four different datasets, including larger numbers of FGR patients and controls from diverse populations, a total of 65 candidate DEGs were identified in FGR versus the control group. Besides, PPI network construction, module analysis of hub genes and validation analysis by microarray data were performed to screen significant hub genes.

In this study, we found four key hub genes (i.e., *CEACAM6*, *DEFA4*, *SCUBE2* and *MPO*) as potential biomarkers for FGR using multiple sequencing studies combining PPI analysis. These four genes were downregulated in samples of GSE75010 which were in accordance with previous observations from four selected placenta RNA-seq data. *CEACAM6* can activate a distinct subset of regulatory CD8 + T cells on placental trophoblasts [26]. These CD8 + regulatory T cells help the mother tolerate her semi-allogeneic fetus [27]. Previous study indicated that maternal-fetal immune tolerance mediated by regulatory T cells played an essential role in the development of FGR [28]. This aberrant expression level of *CEACAM6* may be essential in adjusting placental immunity. *DEFA4* (Defensin alpha 4) has robust features in host defence which gradually increases throughout normal pregnancy [29]. It was suggested that the upregulation of *DEFA*4 [30] in cancer was involved in malignant transformation and correlated with cancer aggressiveness. Trophoblast shares the same properties with cancer cells: tissue invasiveness, immune evasion and stimulation of angiogenesis [31]. Therefore, we conjecture that the placenta cell with low-expressed *DEFA4* loses its aggressive cancer-like manner and led to placenta dysfunction in FGR pathology. *SCUBE2* is identified ubiquitously in vascular endothelium and highly vascularized tissues. Cooperation between the *SCUBE* gene family (*SCUBE1* and *SCUBE2*) regulated angiogenic cell behaviours and formation of functional vessels during zebrafish embryonic development [32]. *MPO* (Myeloperoxidase), an enzyme for activated neutrophils, appeared to be a marker of inflammation and was the core hub gene related to FGR with the highest degree in the current PPI analysis. Previous reports on levels of *MPO* in FGR-related pregnancies were conflicting [33,34]. Higher concentration of *MPO* was seen in the maternal plasma of women with FGR [34]. However, our result showed that *MPO* was downregulated in at least three placenta RNA-seq studies. It seemed that placental *MPO* was unlikely to contribute considerably to the increased levels of plasma *MPO* and inflammatory activation may be confined to the maternal circulation.

Placental vascular pathology is a mechanism of many pregnancy complications, which may cause placental hypoperfusion leading to FGR

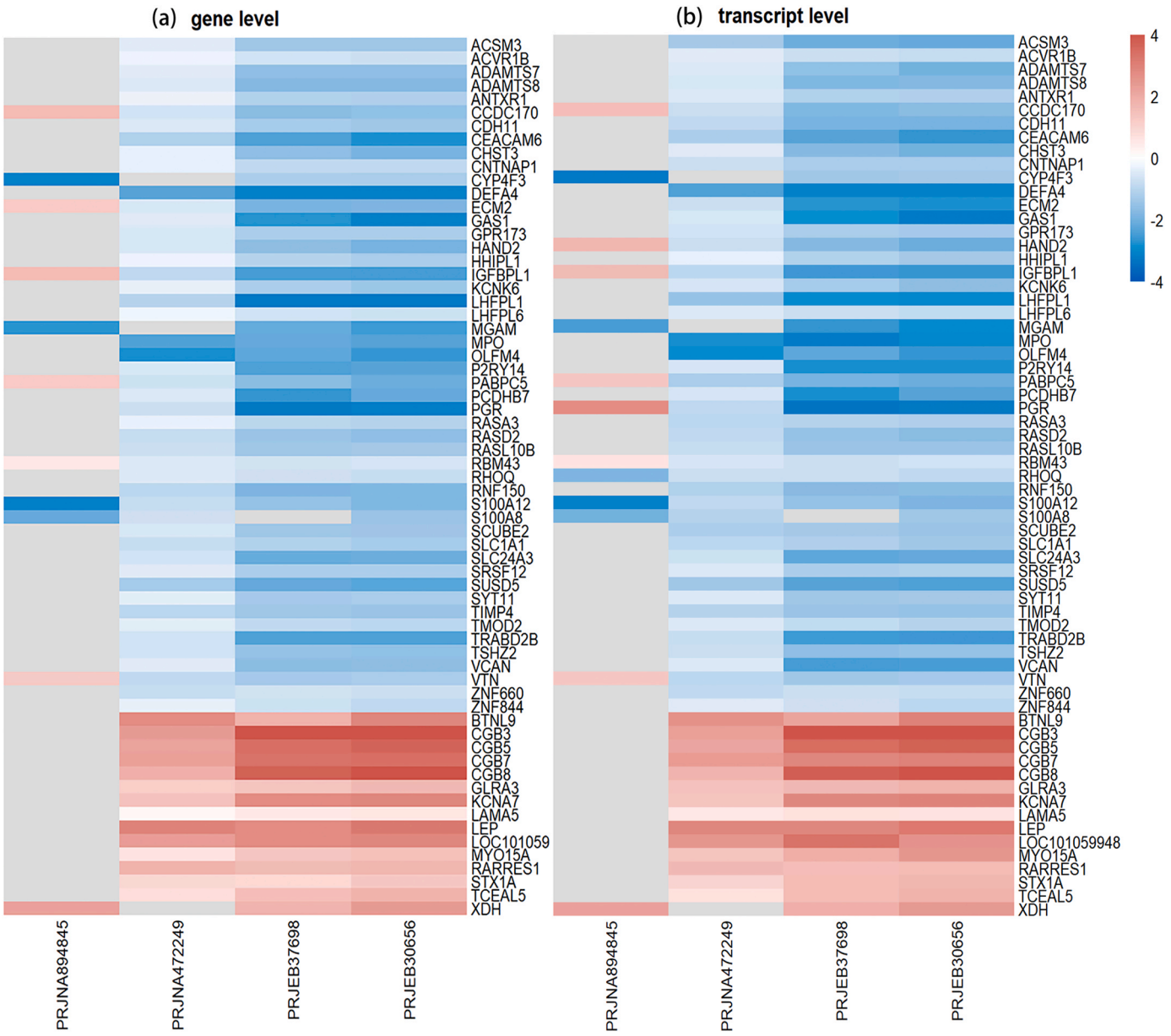

Fig. 2. Heatmap of common differentially expressed genes in FGR analyzed at (a) gene level and (b) transcript level. The color intensity represented log2FoldChange of gene expression by DESeq2. Gray indicated no significant dysregulation.

[35], and affected placentas have increased vascular lesions, especially fetal vascular supply lesions for FGR. In the current analysis, we detected a set of angiogenesis-related genes associated with FGR. *ADAMTSs* (A disintegrin and metalloproteinase with thrombospondin motifs) are a newly defined metalloproteinase family including 19 members involved in vascular biology and diseases. *ADAMTS7* orchestrates functions of vascular smooth muscle cells and endothelial cells to facilitate neointima formation [36]. Knockdown of *ADAMTS7* inhibited the growth and invasion of HTR8/SVneo cells (a trophoblast cell line in early pregnancy) [37]. *VCAN* (Versican) and *VTN* (Vitronectin), which are component of the extracellular matrix, are involved in cell adhesion, proliferation, migration and angiogenesis. Versican silencing in trophoblast cell line induced cell death and affected cell differentiation [38]. Vitronectin regulated trophoblast cells migration and adhesion [39]. Recent studies reported that *VTN* interacted as a downstream signal to vascular endothelial growth factor receptor 2 (VEGFR2) phosphorylation, suggesting their roles in angiogenesis [40]. Given that defective placental angiogenesis is one of the characteristics of FGR [41], we suggest that *ADAMTS7*, *VCAN* and *VTN* may participate in the process of FGR.

S100A12 (S100 calcium-binding protein A12) was downregulated in all four studies at both gene and transcript levels. *S100A12* is mainly expressed in neutrophils, monocytes and macrophages. And, *S100A12* plays a role in several intracellular and extracellular functions, such as immune cell migration, differentiation and inflammation [42,43]. FGR has been linked with abnormal exaggerated maternal inflammatory response [44]. However, the fact that *S100A12* expression was downregulated in FGR placentas may seem paradoxical. Nonetheless, future experiments may provide a possible explanation for this phenomenon.

Endogenous reference genes are routinely used to normalize gene expression levels when confirming the DEGs using alternative methods (e.g., qRT-PCR). However, no housekeeping gene displays stable expression levels under all conditions. In our study, we reported five suitable housekeeper genes within the text of FGR. Our observations on *TBP* and *CYC1* were comparable to previous reports as in [45]. In addition, we also recommend that multiple housekeeping genes be

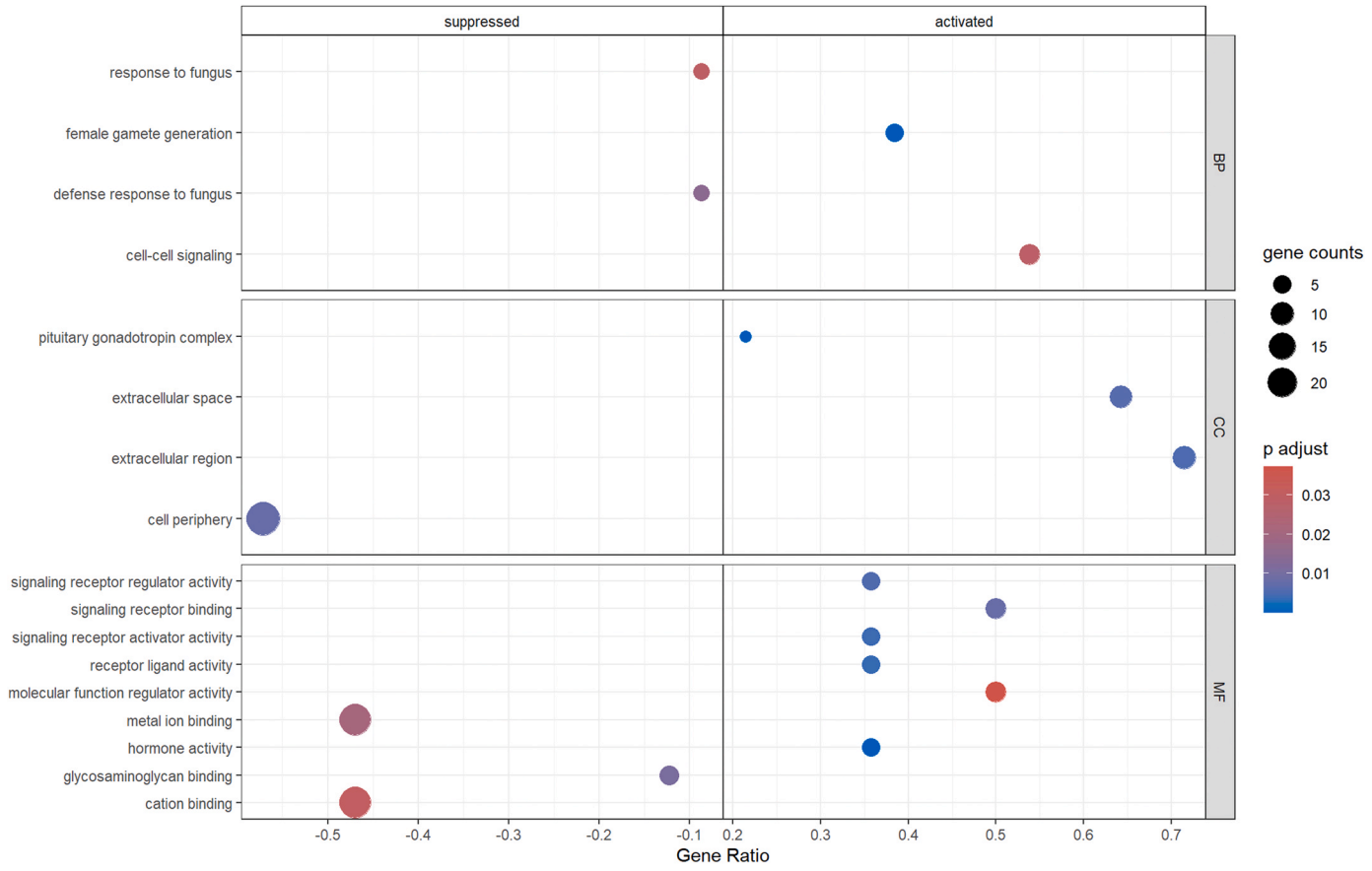

Fig. 3. Gene ontology analysis of 65 candidate DEGs in at least three FGR studies. Significantly affected (p-adjusted value < 0.05) biological processes (BP), cellular components (CC) and molecular functions (MF) were displayed. Dot size indicated the number of genes associated with the functional annotation (gene counts). Gene ratio showed the affected gene counts/size of the corresponding pathway. The right-side showed activated gene ontology terms (Gene ratio > 0); the left-side showed suppressed gene ontology terms (Gene ratio < 0).

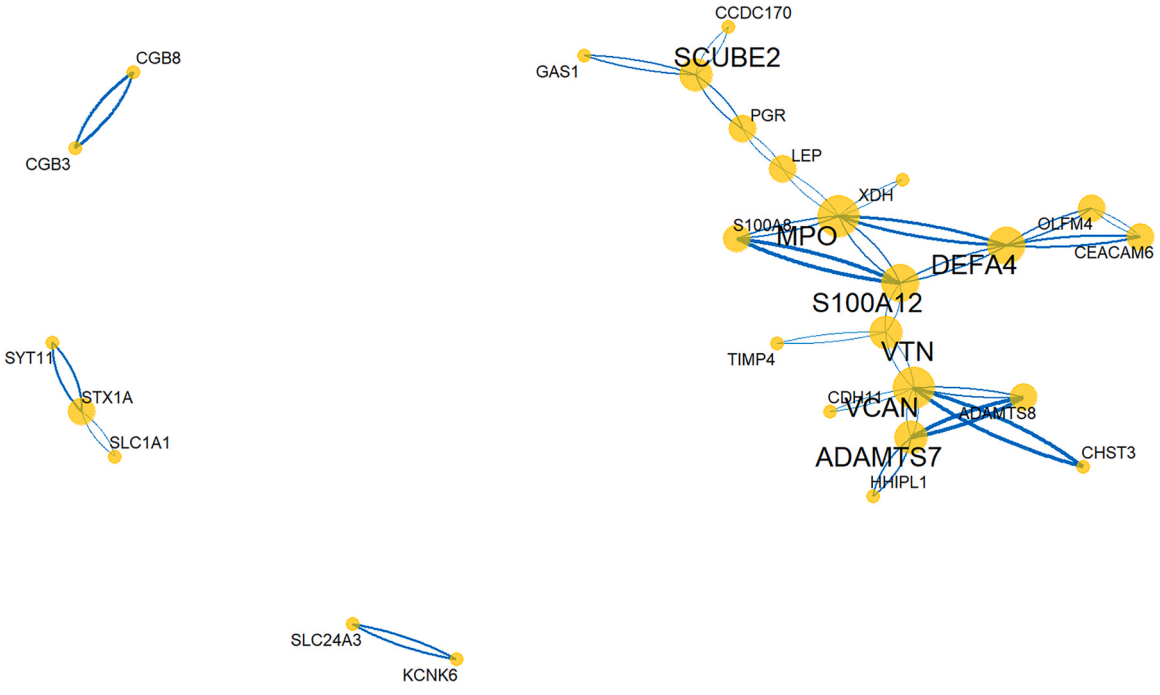

Fig. 4. Network graph of the 65 differentially expressed genes in at least three FGR studies according to the STRING online database. The dots indicated individual differentially expressed genes, and the lines between any nodes represented the interrelations of those proteins.

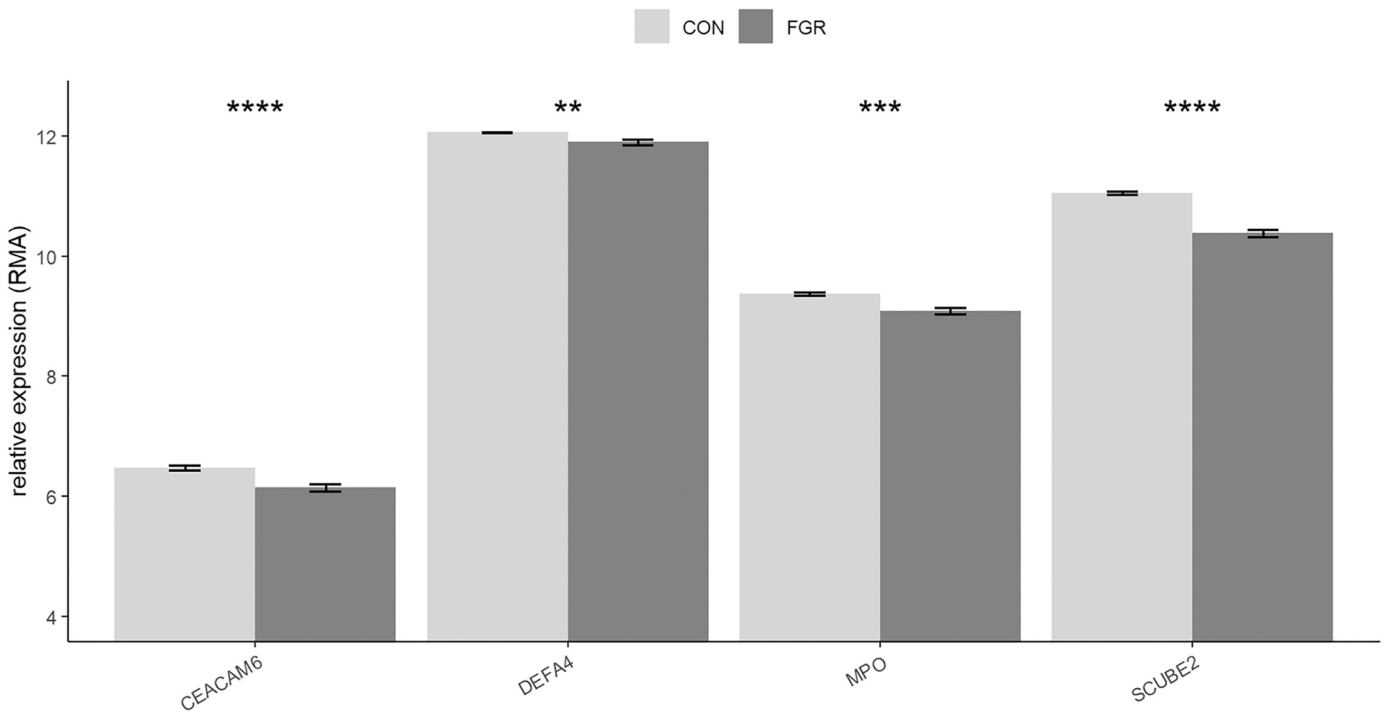

Fig. 5. Validation of the hub genes in microarray dataset GSE75010. The downregulation of the mRNA *CEACAM6*, *DEFA4*, *MPO* and *SCUBE2* in placenta of FGR patients (n = 13) compared to healthy controls (n = 23). Note "****", < 0.0001;"****", < 0.001; "**", p < 0.01.

assessed for better validation of relative gene expression research.

Some limitations of the present study should be acknowledged. First, the validity of our conclusions was mainly based on the established workflow of our combined analysis method and the reliability of the original RNA-seq datasets. Further experimental verification by qRT-PCR or western blot on placenta tissues with larger populations should be performed to validate the hub genes' diagnosis ability. Second, more precise experiments using specific trophoblast cell lines or animal models are needed to explore the function of the hub genes in FGR. Nevertheless, we do think that the combined public data can help guide future marker selection for FGR. Moreover, the clinical data of the patients and newborn children (e.g., maternal age, fetal sex, fetal birth weight and gestation) were either incomplete or not given online. Those confounding variables could not be included or compared within the analysis. We do hope that the data requirements for public databases would be more stringent and complete.

In conclusion, we carried out a cost-effective workflow for initial biomarker screening for FGR based on public data of multiple RNA-seq studies from different backgrounds. Our study identified ten hub genes and three candidate biomarkers that might contribute the development of FGR, which should be helpful for early diagnosis or detection of FGR.

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none.

Author contributions

Xiaohui Li drafted the manuscript and Yi Chen revised the manuscript. Xiaohui Li and Yi Chen conducted data collection and analysis. Xin He and Zhengpeng Li reanalysed the data and revised the manuscript. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author agreement

All authors have read and agreed to the published version of the manuscript.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.eurox.2023.100259.

References

[1] Liu J, Wang XF, Wang Y, Wang HW, Liu Y. The incidence rate, high-risk factors, and short- and long-term adverse outcomes of fetal growth restriction: a report from Mainland China. Medicine 2014;93(27):e210. https://doi.org/10.1097/MD.0000000000000210.

[2] Unterscheider J, O'Donoghue K, Malone FD. Guidelines on fetal growth restriction: a comparison of recent national publications. Am J Perinatol 2015;32(4):307–16. https://doi.org/10.1055/s-0034-1387927.

[3] Brosens I, Pijnenborg R, Vercruysse L, Romero R. The "Great Obstetrical Syndromes" are associated with disorders of deep placentation. Am J Obstet Gynecol 2011;204(3):193–201. https://doi.org/10.1016/j.ajog.2010.08.009.

[4] Malhotra A, Allison BJ, Castillo-Melendez M, Jenkin G, Polglase GR, Miller SL. Neonatal morbidities of fetal growth restriction: pathophysiology and impact. Front Endocrinol 2019;10:55. https://doi.org/10.3389/fendo.2019.00055.

[5] Cox B, Leavey K, Nosi U, Wong F, Kingdom J. Placental transcriptome in development and pathology: expression, function, and methods of analysis. Am J

Obstet Gynecol 2015;213(4 Suppl):S138–51. https://doi.org/10.1016/j.ajog.2015.07.046.
[6] Stepan H, Galindo A, Hund M, et al. Clinical utility of sFlt-1 and PlGF in screening, prediction, diagnosis and monitoring of pre-eclampsia and fetal growth restriction. Ultrasound Obstet Gynecol 2022. https://doi.org/10.1002/uog.26032.
[7] Smith GCS. Developing novel tests to screen for fetal growth restriction. Trends Mol Med 2021;27(8):743–52. https://doi.org/10.1016/j.molmed.2021.05.006.
[8] King VJ, Bennet L, Stone PR, Clark A, Gunn AJ, Dhillon SK. Fetal growth restriction and stillbirth: biomarkers for identifying at risk fetuses. Front Physiol 2022;13:959750. https://doi.org/10.3389/fphys.2022.959750.
[9] Smith GCS. Universal screening for foetal growth restriction. Best Pr Res Clin Obstet Gynaecol 2018;49:16–28. https://doi.org/10.1016/j.bpobgyn.2018.02.008.
[10] Gong S, Gaccioli F, Dopierala J, et al. The RNA landscape of the human placenta in health and disease. Nat Commun 2021;12(1):2639. https://doi.org/10.1038/s41467-021-22695-y.
[11] Yong HEJ, Chan SY. Current approaches and developments in transcript profiling of the human placenta. Hum Reprod Update 2020;26(6):799–840. https://doi.org/10.1093/humupd/dmaa028.
[12] Rezk M, Grasegger L, Brandstetter N, et al. Biomarker screening in preeclampsia: an RNA-sequencing approach based on data from multiple studies. J Hypertens 2022;40(10):2022–36. https://doi.org/10.1097/HJH.0000000000003226.
[13] Majewska M, Lipka A, Paukszto L, et al. Placenta Transcriptome Profiling in Intrauterine Growth Restriction (IUGR). Int J Mol Sci 2019;20(6). https://doi.org/10.3390/ijms20061510.
[14] Lipka A, Jastrzebski JP, Paukszto L, et al. Sex-Biased lncRNA signature in fetal growth restriction (FGR). Cells 2021;10(4):921. https://doi.org/10.3390/cells10040921.
[15] Awamleh Z, Gloor GB, Han VKM. Placental microRNAs in pregnancies with early onset intrauterine growth restriction and preeclampsia: potential impact on gene expression and pathophysiology. BMC Med Genom 2019;12(1):91. https://doi.org/10.1186/s12920-019-0548-x.
[16] Chen S, Zhou Y, Chen Y, Gu J. fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics 2018;34(17):i884–90. https://doi.org/10.1093/bioinformatics/bty560.
[17] Kim D, Paggi JM, Park C, Bennett C, Salzberg SL. Graph-based genome alignment and genotyping with HISAT2 and HISAT-genotype. Nat Biotechnol 2019;37(8):907–15. https://doi.org/10.1038/s41587-019-0201-4.
[18] Danecek P, Bonfield JK, Liddle J, et al. Twelve years of SAMtools and BCFtools. Gigascience 2021;10(2). https://doi.org/10.1093/gigascience/giab008.
[19] Liao Y, Smyth GK, Shi W. featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. Bioinformatics 2014;30(7):923–30. https://doi.org/10.1093/bioinformatics/btt656.
[20] Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 2014;15(12):550. https://doi.org/10.1186/s13059-014-0550-8.
[21] Raudvere U, Kolberg L, Kuzmin I, et al. g:Profiler: a web server for functional enrichment analysis and conversions of gene lists (2019 update). Nucleic Acids Res 2019;47(W1):W191–8. https://doi.org/10.1093/nar/gkz369.
[22] Shannon P, Markiel A, Ozier O, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 2003;13(11):2498–504. https://doi.org/10.1101/gr.1239303.
[23] Wolter M, Rower C, Koy C, Rath W, Pecks U, Glocker MO. Proteoform profiling of peripheral blood serum proteins from pregnant women provides a molecular IUGR signature. J Proteom 2016;149:44–52. https://doi.org/10.1016/j.jprot.2016.04.027.
[24] Sovio U, Goulding N, McBride N, et al. A maternal serum metabolite ratio predicts fetal growth restriction at term. Nat Med 2020;26(3):348–53. https://doi.org/10.1038/s41591-020-0804-9.
[25] Gurugubelli Krishna R, Bhat BV, Bobby Z, Papa D, Badhe B, Chinnakali P. Are Global DNA methylation and telomere length useful biomarkers for identifying intrauterine growth restricted neonates? J Matern Fetal Neonatal Med 2021;34(5):761–4. https://doi.org/10.1080/14767058.2019.1615875.
[26] Shao L, Allez M, Park MS, Mayer L. Immunomodulatory roles of the carcinoembryonic antigen family of glycoproteins. Ann N Y Acad Sci 2006;1072:194–209. https://doi.org/10.1196/annals.1326.037.
[27] Shao L, Jacobs AR, Johnson VV, Mayer L. Activation of CD8+ regulatory T cells by human placental trophoblasts. J Immunol 2005;174(12):7539–47. https://doi.org/10.4049/jimmunol.174.12.7539.
[28] Xu Y, Su M, Wang Z, et al. Dysfunction of Tregs contributes to FGR pathogenesis via regulating Smads signalling pathway. J Cell Mol Med 2020;24(6):3647–55. https://doi.org/10.1111/jcmm.15059.
[29] Gomez-Lopez N, Romero R, Hassan SS, et al. The cellular transcriptome in the maternal circulation during normal pregnancy: a longitudinal study. Front Immunol 2019;10:2863. https://doi.org/10.3389/fimmu.2019.02863.
[30] Basingab F, Alsaiary A, Almontashri S, et al. Alterations in Immune-Related Defensin Alpha 4 (DEFA4) gene expression in health and disease. Int J Inflam 2022;2022:9099136. https://doi.org/10.1155/2022/9099136.
[31] Costanzo V, Bardelli A, Siena S, Abrignani S. Exploring the links between cancer and placenta development. Open Biol 2018;8(6). https://doi.org/10.1098/rsob.180081.
[32] Tsao KC, Lin YC, Chen YT, Lai SL, Yang RB. Zebrafish Scube1 and Scube2 cooperate in promoting Vegfa signalling during embryonic vascularization. Cardiovasc Res 2022;118(4):1074–87. https://doi.org/10.1093/cvr/cvab125.
[33] Hung TH, Chen SF, Lo LM, Li MJ, Yeh YL, Hsieh TT. Myeloperoxidase in the plasma and placenta of normal pregnant women and women with pregnancies complicated by preeclampsia and intrauterine growth restriction. Placenta 2012;33(4):294–303. https://doi.org/10.1016/j.placenta.2012.01.004.
[34] Thaete LG, Qu XW, Neerhof MG, Hirsch E, Jilling T. Fetal growth restriction induced by transient uterine ischemia-reperfusion: differential responses in different mouse strains. Reprod Sci 2018;25(7):1083–92. https://doi.org/10.1177/1933719117732160.
[35] Kovo M, Schreiber L, Bar J. Placental vascular pathology as a mechanism of disease in pregnancy complications. Thromb Res 2013;131(Suppl 1):S18–21. https://doi.org/10.1016/S0049-3848(13)70013-6.
[36] Kessler T, Zhang L, Liu Z, et al. ADAMTS-7 inhibits re-endothelialization of injured arteries and promotes vascular remodeling through cleavage of thrombospondin-1. Circulation 2015;131(13):1191–201. https://doi.org/10.1161/CIRCULATIONAHA.114.014072.
[37] Meng YH, Zhang JB, Sun YL, Liu XL. ADATMS-7 regulates the focal adhesion kinase signaling and promotes invasiveness of trophoblasts in early pregnancy. Placenta 2020;92:54–61. https://doi.org/10.1016/j.placenta.2020.02.010.
[38] Pires KSN, Sun SY, Goncalves CM, et al. Versican silencing in BeWo cells and its implication in gestational trophoblastic diseases. Histochem Cell Biol 2019;151(4):305–13. https://doi.org/10.1007/s00418-018-1739-9.
[39] Douglas GC, Thirkill TL, Blankenship TN. Vitronectin receptors are expressed by macaque trophoblast cells and play a role in migration and adhesion to endothelium. Biochim Biophys Acta 1999;1452(1):36–45. https://doi.org/10.1016/s0167-4889(99)00109-3.
[40] Lian L, Li XL, Xu MD, et al. VEGFR2 promotes tumorigenesis and metastasis in a pro-angiogenic-independent way in gastric cancer. BMC Cancer 2019;19(1):183. https://doi.org/10.1186/s12885-019-5322-0.
[41] Su EJ, Xin H, Yin P, et al. Impaired fetoplacental angiogenesis in growth-restricted fetuses with abnormal umbilical artery doppler velocimetry is mediated by aryl hydrocarbon receptor nuclear translocator (ARNT). J Clin Endocrinol Metab 2015;100(1):E30–40. https://doi.org/10.1210/jc.2014-2385.
[42] Donato R, Cannon BR, Sorci G, et al. Functions of S100 proteins. Curr Mol Med 2013;13(1):24–57.
[43] Xia C, Braunstein Z, Toomey AC, Zhong J, Rao X. S100 Proteins as an important regulator of macrophage inflammation. Front Immunol 2017;8:1908. https://doi.org/10.3389/fimmu.2017.01908.
[44] Cotechini T, Graham CH. Aberrant maternal inflammation as a cause of pregnancy complications: a potential therapeutic target? Placenta 2015;36(8):960–6. https://doi.org/10.1016/j.placenta.2015.05.016.
[45] Drewlo S, Levytska K, Kingdom J. Revisiting the housekeeping genes of human placental development and insufficiency syndromes. Placenta 2012;33(11):952–4. https://doi.org/10.1016/j.placenta.2012.09.007.