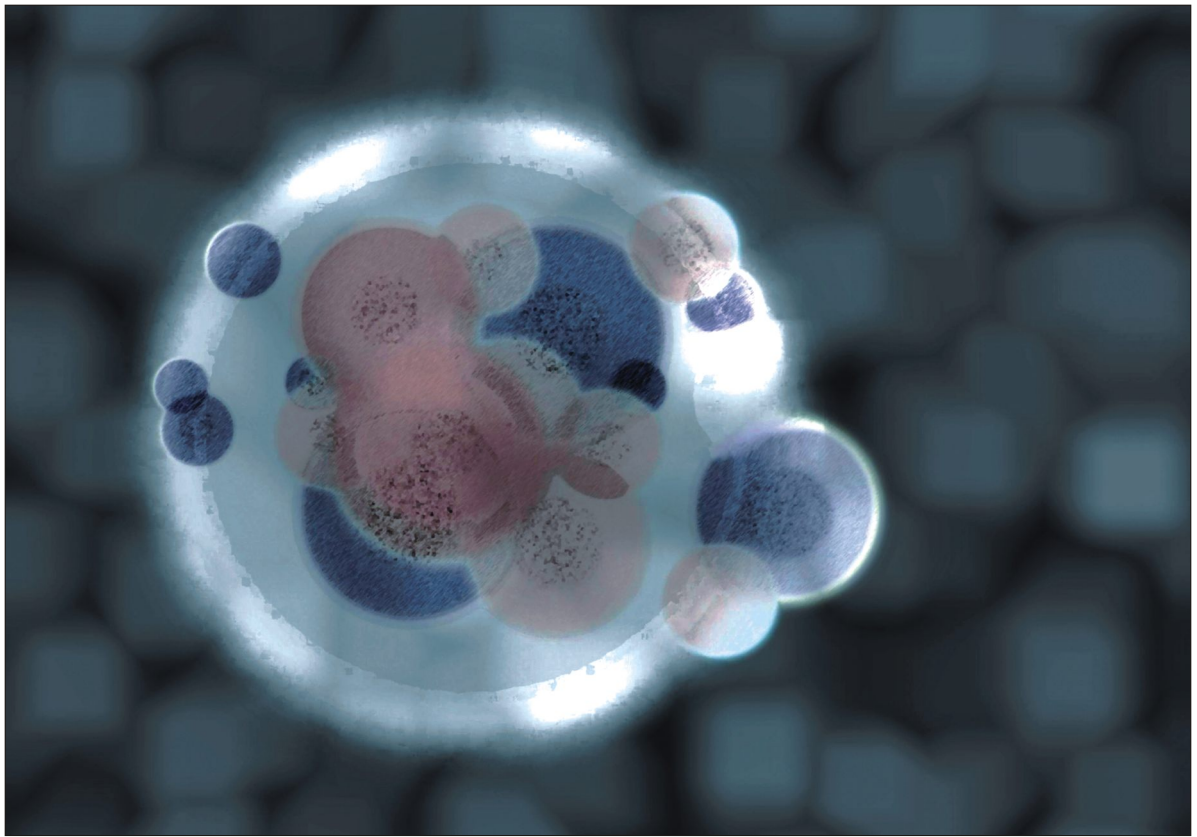




Journal of Biological Sciences

ISSN 1727-3048

science
alert

ANSI*net*
an open access publisher
<http://ansinet.com>



Research Article

Analyzing Expression of GPCRs in Healthy and Preeclamptic Human Placenta

¹Rafia Aziz, ²Afak Yusuf Sherwani and ³Sameer Mohammad

¹Department of Gynecology, Government Medical College Baramulla, Baramulla, Jammu and Kashmir, India

²Department of General Surgery, Government Medical College Baramulla, Baramulla, Jammu and Kashmir, India

³King Abdullah International Medical Research Center, Department of Experimental Medicine, King Saud bin Abdulaziz University for Health Sciences (Ministry of National Guard Health Affairs), Riyadh 11426, Saudi Arabia

Abstract

Background and Objective: Preeclampsia is one of the most dangerous hypertensive disorders that may arise during pregnancy and it is a major cause of maternal and fetal morbidity and mortality. Despite being a prominent topic of scientific inquiry, the fundamental pathways that contribute to the pathophysiology of preeclampsia remain little understood. Recent research suggested that G Protein-Coupled Receptors (GPCRs) may play a role in the etiology of preeclampsia, certain of these receptors have been observed to be elevated during a healthy pregnancy and decreased in preeclamptic people. However, the expression and functional importance of the great majority of GPCRs in the preeclamptic placenta are unclear. The goal of this study was to find highly expressed GPCRs that might play key roles in the physiology and pathology of the placenta. **Materials and Methods:** Dataset GSE148241 contained RNA sequencing data of 41 placentae in total, including from 9 patients with early-onset severe preeclampsia (EOSPE) and 32 normal controls. The RNA sequencing data were re-analyzed to evaluate the mRNA expression of the whole set of non-viral GPCRs in normal and preeclamptic human placenta. **Results:** The RNA sequencing identified more than 200 GPCRs in the healthy human placenta. Adhesion receptors (ADRG6 and ADGRG1), Atypical Chemokine Receptor 2 (ACKR2), Melanocortin Receptor 1 (MC1R), GPR107 and GPR78 are among the most abundant GPCRs. The expression of several GPCRs is significantly altered in the preeclamptic placenta. The list of GPCRs that are upregulated includes CCR5, HCAR2, GPR32, ADORA2A and GPR17 and those that are downregulated include ACKR1, SSTR1, OPRK1, FPR3 and CX3CR1. **Conclusion:** The GPCRs with altered expression in the preeclamptic may have substantial physiological importance in the placenta and may bring novel therapeutic options.

Key words: G protein-coupled receptors, pregnancy, hypertension, preeclampsia, RNA sequencing, gene expression, fetal health

Citation: Aziz, R., A.Y. Sherwani and S. Mohammad, 2023. Analyzing expression of GPCRs in healthy and preeclamptic human placenta. J. Biol. Sci., 23: 1-9.

Corresponding Author: Sameer Mohammad, King Abdullah International Medical Research Center, Department of Experimental Medicine, King Saud bin Abdulaziz University for Health Sciences (Ministry of National Guard Health Affairs), Riyadh 11426, Saudi Arabia

Copyright: © 2023 Rafia Aziz *et al.* This is an open access article distributed under the terms of the creative commons attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Preeclampsia (PE) is a serious illness that develops after 20 weeks of pregnancy and is defined by newly developed hypertension, proteinuria, uteroplacental insufficiency or damage to the mother's organs¹. Women with PE experience a wide range of indications and symptoms related to many organ systems, including headache, thrombocytopenia, chest tightness, pulmonary edema, reduced oxygen saturation, severe hypertension and reduced liver and kidney function. This disease is a leading cause of maternal and fetal morbidity and mortality^{2,3}. Many research investigations have focused on the pathophysiological mechanisms of PE^{4,5}. The placenta is the primary determinant in the development of PE. All maternal difficulties are characterized by a common pathophysiological component that focuses on placental anomalies³. Poor placentation and endothelial cell dysfunction all contribute to the etiology of PE. The molecular mechanism behind PE is still unknown and there has been little progress in its management due to the disease's variability and the wide range of clinical symptoms associated with it⁵⁻⁷. The G Protein-Coupled Receptors (GPCRs), the largest class of membrane proteins in eukaryotes, regulate virtually all life processes and functions⁸. The GPCRs are activated by a wide variety of ligands or stimuli, including hormones, neurotransmitters and light, to regulate key physiological processes. The GPCRs are important factors in the onset and progression of various diseases and many marketed drugs exert their efficacy through GPCR signaling^{9,10}. Exploratory studies have highlighted the critical roles of GPCRs in placental development and offered a theoretical foundation for their usage as prospective PE targets^{11,12}. The GPCRs that have been studied for their role in placental development include Calcitonin Receptor (CALCR), Parathyroid Hormone 1 Receptor (PTH1R), Corticotropin-Releasing Hormone Receptor (CRHR1 and CRHR2), Glucagon Receptor (GCGR), Glucagon Like Peptide Receptor 1 (GLP1R) and adhesion receptors (ADGRA2, ADGRF5, ADGRL4, ADGRG6, ADGRE5 and ADGRG2)¹³⁻¹⁶. However, the expression and functional significance of a vast majority of GPCRs in placental biology are unclear. The RNA sequencing data was re-analyzed to assess the mRNA expression of the entire set of non-visual GPCRs. The RNA sequencing detected more than 200 GPCRs in the healthy human placenta and the expression of multiple GPCRs is significantly altered in the preeclamptic placenta. The GPCRs with altered expression in the diseased placenta may have significant physiological importance in the placenta and may provide novel treatment approaches.

MATERIALS AND METHODS

Study area: The study was carried out in Government Medical College Baramulla, Baramulla, Kashmir, India and King Abdullah International Medical Research Riyadh, Saudi Arabia.

Datasets: The RNA sequencing datasets GSE77085 and GSE148241 were used in this study. This dataset GSE77085 contained RNA sequencing data of villous tissue from 16 human placental samples. Third-trimester placenta samples taken from primiparous women with singleton pregnancies were used in this study¹⁷. Dataset GSE148241 contained RNA sequencing data of 41 placentae in total, including from 9 patients with early-onset severe preeclampsia (EOSPE) and 32 normal controls¹⁸.

RNA sequencing re-analysis: The RNA sequencing datasets GSE77085 and GSE148241 were analyzed by the GEO RNA-seq Experiments Interactive Navigator (GREIN) platform¹⁹. The CPM-normalized mRNA expression was obtained from the GREIN platform for GSE77085 and the mean expression of each gene was determined. Dataset GSE148241 was analyzed on GREIN to determine differential gene expression between EOSPE and control placentae. Data were downloaded and the expression of the entire set of non-visual GPCRs (as per the International Union of Basic and Clinical Pharmacology Committee on Receptor Nomenclature and Drug Classification guidelines) was extracted TBTools software^{20,21}.

Statistical analysis: The GPCR mRNA expression for normal placentae was expressed as Average $\pm$ SEM. For comparison of mRNA expression between EOSPE and normal placentae, adjusted p-values (obtained from GREIN) were used and considered significant at $p < 0.01$.

RESULTS

Landscape of the GPCRs in the human placenta: Expression of the entire repertoire of non-visual GPCRs in the human placenta was determined by reanalysis of the GSE77085 RNA sequencing dataset. The dataset contained RNA sequencing data from 16 human placentas and was available on the GEO RNA-seq experiments interactive navigator (GREIN) platform. Normalized gene expression data were downloaded from GREIN and data for all GPCRs were extracted using

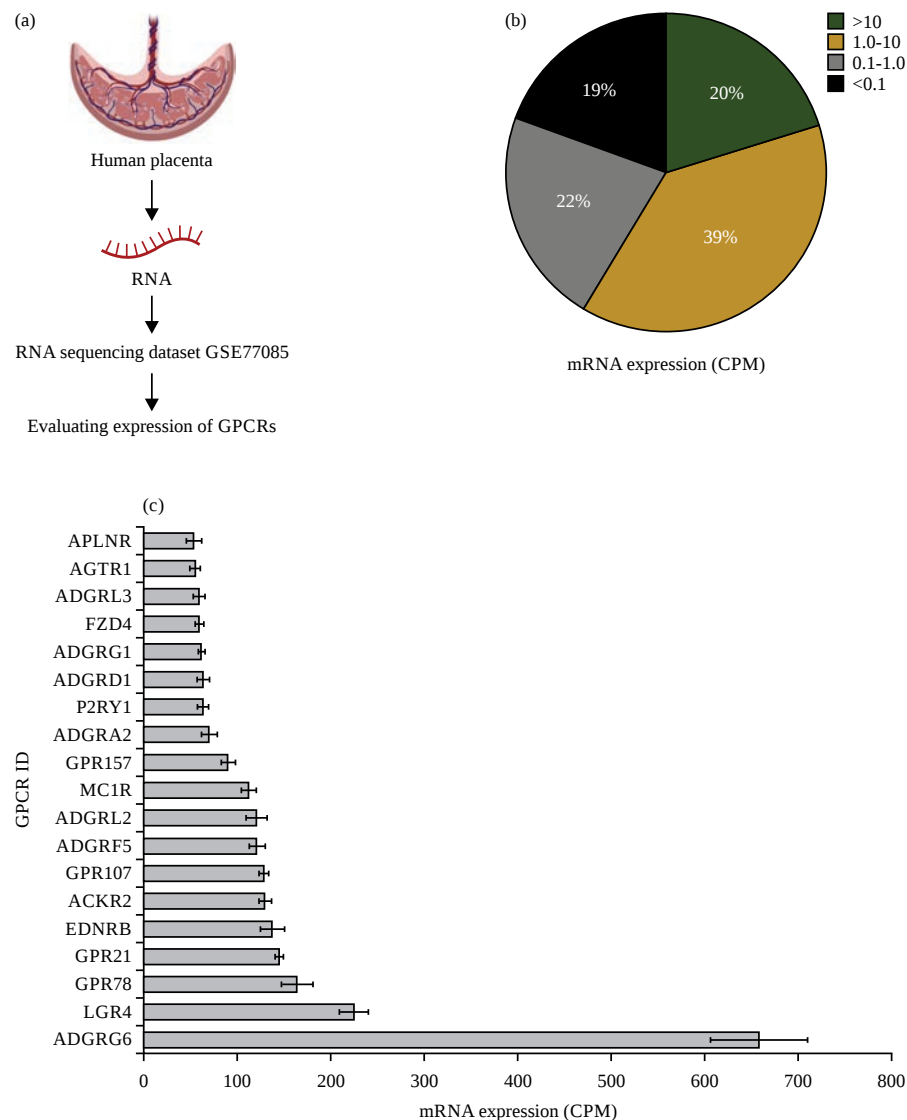


Fig. 1(a-c): mRNA expression profile of GPCRs in healthy human placenta, (a) Outline of the analysis, (b) Pi-chart showing the distribution of GPCRs based on their mRNA expression and (c) List of the top 20 highly expressed GPCRs

Data was extracted from GSE77085 containing RNA sequencing data of 16 human placentae. The data for each GPCR is expressed as normalized CPM $\pm$ SEM

Tbtools (Fig. 1a). A total of 291 GPCRs were detected in the human placenta of which 73 were highly expressed (CPM>10), 139 were moderately expressed (CPM 1-10) and 79 were expressed at low levels (CPM 0.1-1) (Fig. 1b). Since 70 GPCRs had a CPM of 0.1 or were not detected, they were regarded as absent. The average expression of all the detected GPCRs was 14.2 CPM and 50 GPCRs were expressed at levels higher than the average expression (Table 1). Members of the adhesion receptor family (ADGRG6, ADGRF5, ADGRA2, ADGRD1, ADGRG1, ADGRL3 and ADGRL2), orphan receptors (GPR78, GPR21, GPR107 and GPR157), Endothelin Receptor

Type B (EDNRB), Atypical Chemokine Receptor 2 (ACKR2), Melanocortin Receptor 1 (MC1R), Purinergic Receptor P2Y1 (P2RY1), Frizzled Class Receptor 4 (FZD4), Angiotensin II Receptor Type 1 (AGTR1) and Apelin Receptor (APLNR) were among the highly expressed GPCRs in human placenta (Fig. 1c).

Impact of preeclampsia on GPCRs placental GPCR expression profile: Dataset GSE148241 contains RNA sequencing data from 41 human placentas, 32 from normal controls and 9 from patients with Early-Onset Severe

Table 1: List of GPCRs with higher levels of expression than the global average

Gene symbol	Gene name	CPM
ADGRG6	Adhesion G protein-coupled receptor G6	656.93
LGR4	Leucine rich repeat containing G-protein-coupled receptor 4	224.29
GPR78	G protein-coupled receptor 78	163.55
GPR21	G protein-coupled receptor 21	144.49
EDNRB	Endothelin receptor type B	136.99
ACKR2	Atypical chemokine receptor 2	129.56
GPR107	G protein-coupled receptor 119	127.96
ADGRF5	Adhesion G protein-coupled receptor F5	121.27
ADGRL2	Adhesion G protein-coupled receptor L2	120.39
MC1R	Melanocortin 1 receptor	111.90
GPR157	G protein-coupled receptor 157	90.07
ADGRA2	Adhesion G protein-coupled receptor A2	69.66
P2RY1	Purinergic receptor P2Y1	63.00
ADGRD1	Adhesion G protein-coupled receptor D1	62.95
ADGRG1	Adhesion G protein-coupled receptor G1	61.35
FZD4	Frizzled class receptor 4	59.23
ADGRL3	Adhesion G protein-coupled receptor L3	58.88
AGTR1	Angiotensin II receptor type 1	54.70
APLNR	Apelin receptor	53.36
ADGRE5	Adhesion G protein-coupled receptor E5	49.26
OPN3	Opsin 3	47.91
ADGRA3	Adhesion G protein-coupled receptor A3	42.24
P2RY6	Pyrimidinergic receptor P2Y6	39.97
S1PR3	Sphingosine-1-phosphate receptor 3	38.06
S1PR1	Sphingosine-1-phosphate receptor 1	37.08
LPAR6	Lysophosphatidic acid receptor 6	36.90
ADRB1	Adrenoceptor beta 1	33.05
LGR5	Leucine rich repeat containing G protein-coupled receptor 5	31.48
FZD6	Frizzled class receptor 6	26.64
LPAR1	Lysophosphatidic acid receptor 1	26.58
CMKLR1	Chemerin chemokine-like receptor 1	26.24
CYSLTR1	Cysteinyl leukotriene receptor 1	26.15
FZD1	Frizzled class receptor 1	25.48
P2RY14	Purinergic receptor P2Y14	24.76
S1PR2	Sphingosine-1-phosphate receptor 2	24.38
TPRA1	Transmembrane protein adipocyte associated 1	23.03
GPR137	G protein-coupled receptor 137	23.01
GPR34	G protein-coupled receptor 34	20.35
CYSLTR2	Cysteinyl leukotriene receptor 2	18.58
ADGRF1	Adhesion G protein-coupled receptor F1	18.08
FZD7	Frizzled class receptor 7	18.01
ACKR3	Atypical chemokine receptor 3	17.72
SSTR2	Somatostatin receptor 2	16.40
FZD5	Frizzled class receptor 5	16.29
OPRK1	Opioid receptor kappa 1	16.29
CSAR2	Complement C5a receptor 2	16.00
CALCRL	Calcitonin receptor like receptor	15.84
ADGRL4	Adhesion G protein-coupled receptor L4	15.40
CXCR4	C-X-C motif chemokine receptor 4	15.24
FPR1	Formyl peptide receptor 1	14.36

Information is presented as normalized CPM (mean of 16 healthy human placentae from RNA sequencing dataset GSE77085)

Preeclampsia (EOSPE). The data was processed using the GREIN platform and a list of differentially expressed genes between preeclamptic and normal placentae was generated (Fig. 2a). The data for all GPCRs were extracted using TBtools. Preeclampsia had a substantial effect on the

expression of GPCRs and several members of this family show significant changes in their expression in preeclamptic placenta (Fig. 2b). Twenty two GPCRs were upregulated and 9 GPCRs were downregulated in the preeclamptic placenta ($p_{adj} < 0.01$). The highly upregulated GPCRs include

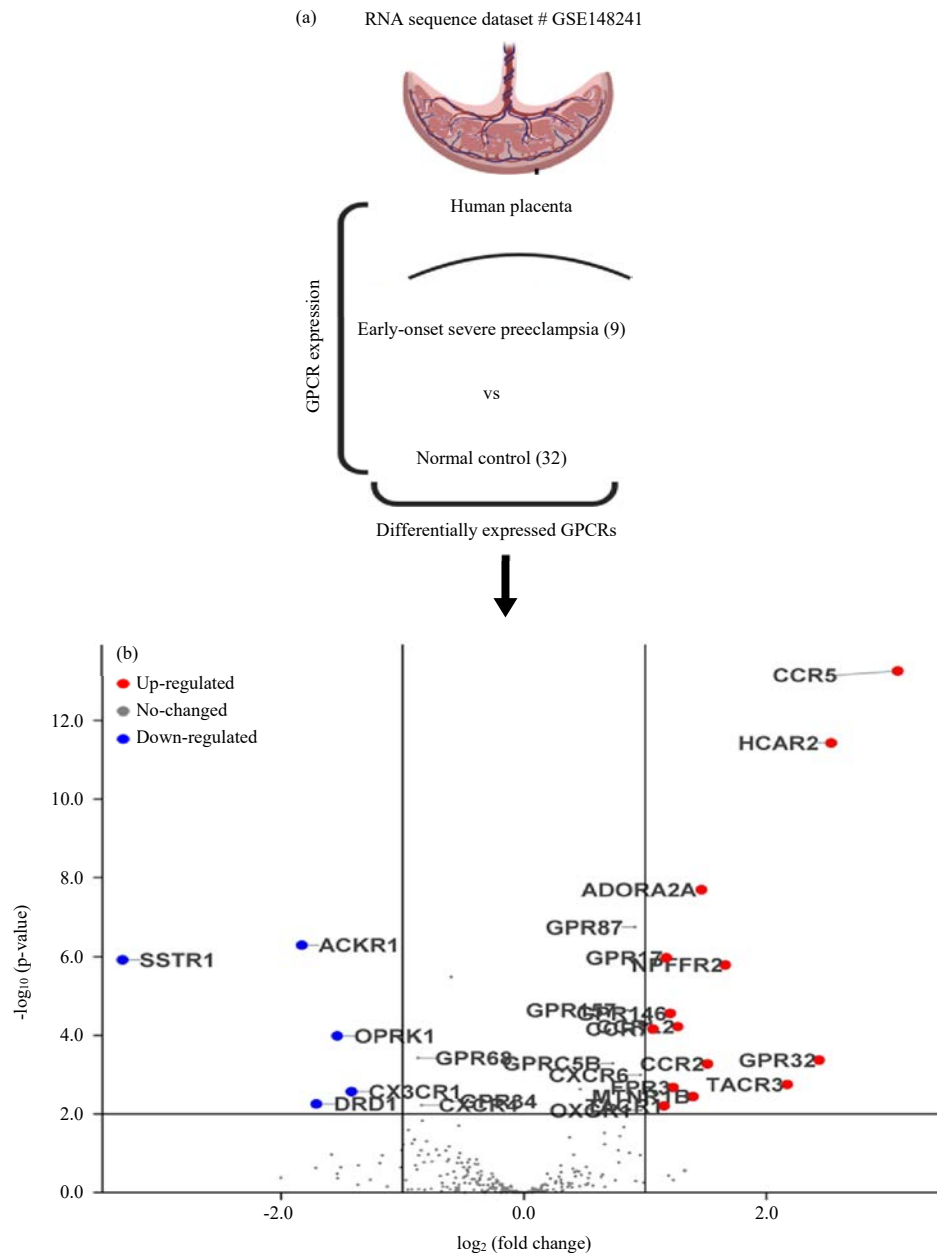


Fig. 2(a-b): Differential expression of GPCRs in preeclamptic placentae compared to normal healthy controls, (a) Framework of the analysis and (b) Volcano plot showing the differential expression of GPCRs between preeclamptic and normal placentae

Differential expression of genes was obtained by re-analyzing RNA sequencing dataset # GSE148241, which contains data from placentae from 9 preeclampsia patients and 32 normal controls. The highlighted genes show a twofold or greater change and statistical significance (adjusted $p < 0.01$)

Chemokine receptor (CCR5), Hydroxycarboxylic Acid Receptor 2 (HCAR2), uracil nucleotide/cysteinyl leukotriene receptor (GPR17), resolving receptor (GPR32), Melatonin Receptor 1B (MTNR1B) and adenosine A2A Receptor (ADORA2A) (Fig. 3a-f). Downregulated GPCRs include Atypical

Chemokine Receptor 1 (ACKR1), Kappa Opioid Receptor (OPRK1), Somatostatin Receptor 1 (SSTR1), C-X-C Chemokine Receptor Type 5 (CXCR5), pH-Sensing G Protein-Coupled Receptor (GPR68) and C-X-C chemokine receptor type 4 (CXCR4) (Fig. 4a-f).

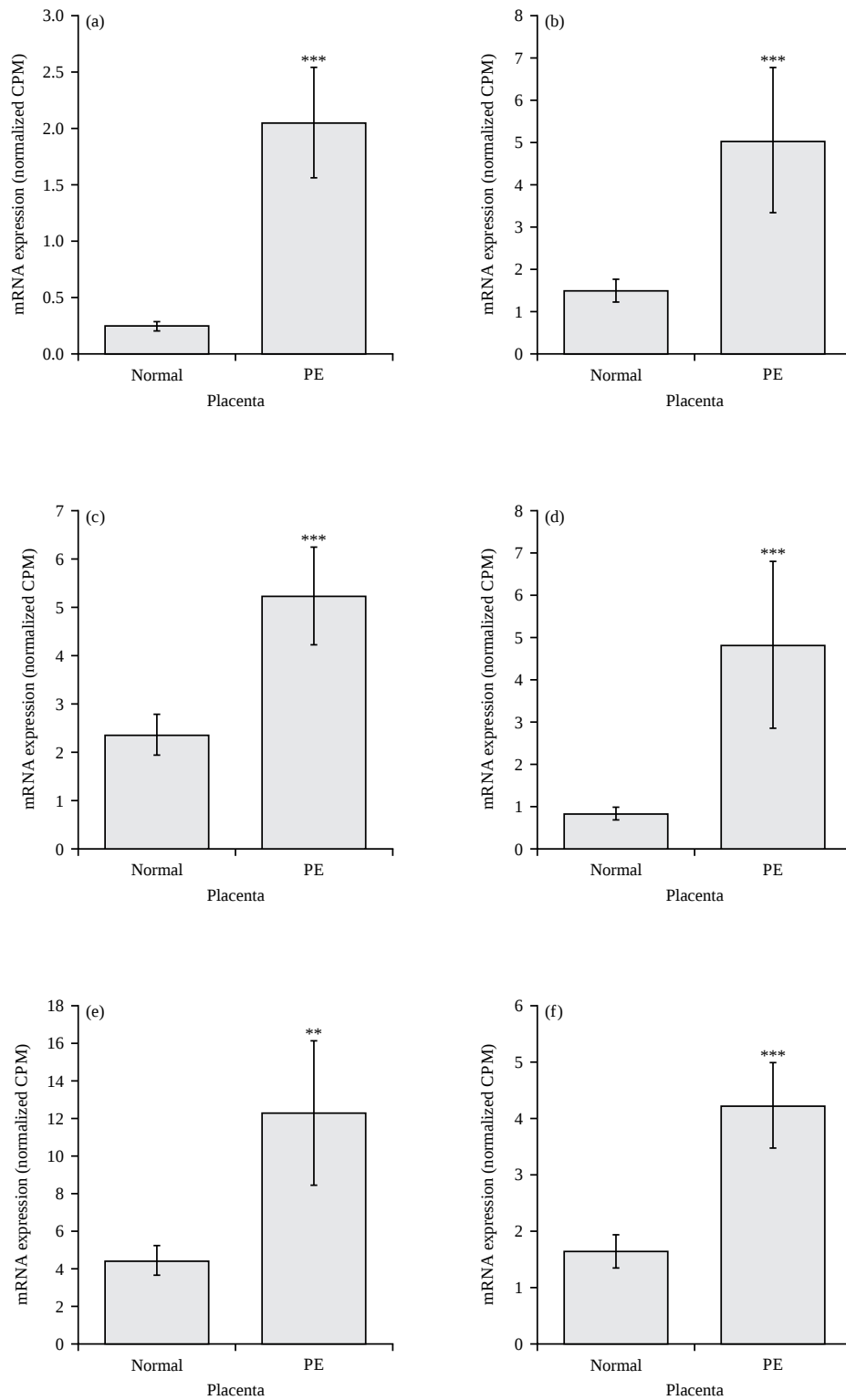


Fig. 3(a-f): GPCRs upregulated in preeclamptic placentae compared to normal healthy controls, (a) CCR5, (b) HCAR2, (c) GPR17, (d) GPR32, (e) MTNR1B and (f) ADORA2A

Normalized CPM of indicated genes in placenta from normal (N = 32) and preeclampsia patients (N = 9), each bar represents the average CPM ± SEM, adjusted p-values ***p<0.001 and **p<0.01

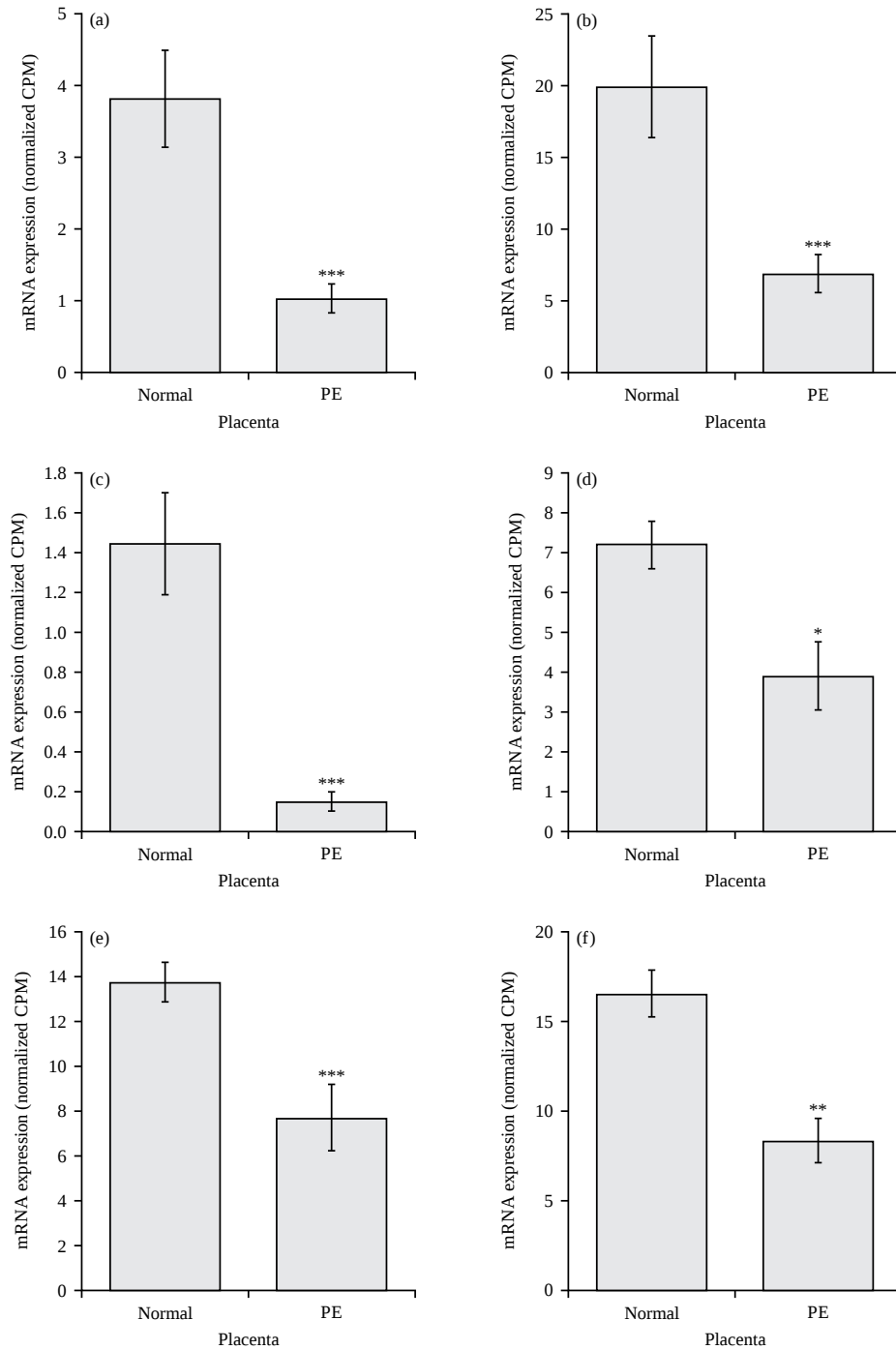


Fig.4(a-f): GPCRs with reduced expression in preeclamptic placentae compared to normal healthy controls, (a) ACKR1, (b) OPRK1, (c) SSTR1, (d) CXCR5, (e) GPR68 and (f) CXCR4

Normalized CPM of indicated genes in placenta from normal (N = 32) and preeclampsia patients (N = 9), each bar represents the average CPM $\pm$ SEM, adjusted p-values ***p<0.001, **p<0.01 and *p<0.05

DISCUSSION

Preeclampsia (PE) is a multisystem pregnancy condition that causes new-onset hypertension after 20 weeks of gestation and impairs several organ systems. The PE affects

around 5-8% of pregnant women and is a major cause of maternal mortality^{4,5}. Women with preeclampsia are more likely to experience subsequent health issues in addition to negative pregnancy outcomes. The immunological, genetic and environmental factors behind PE are still not completely

known and there hasn't been a significant advancement in PE therapy as a result of the heterogeneity of PE and the variety of its clinical presentations^{22,23}. The GPCRs are a class of seven-transmembrane receptors that make up the largest group of cell surface receptors in mammalian cells. They control several crucial biological processes and abnormal GPCR signalling has been linked to the pathophysiology of several illnesses. The GPCRs are appealing therapeutic targets due to their unique combination of variation and specificity within the GPCR family and the fact that they are easily targetable by exogenous drugs. Several studies have shown the importance of GPCRs in placental development and provided a theoretical basis for their use as prospective PE targets^{11,24,25}. However, the expression and functional importance of a vast majority of GPCRs in placental biology are unknown. This is in part, due to the scarcity of expression studies. Therefore, this study was initiated to comprehensively profile the entire repertoire of non-visual GPCRs by re-analyzing RNA-sequencing data. A total of 291 GPCRs were detected in the human placenta of which 73 were highly expressed (CPM>10). Many of the highly expressed GPCRs have no known role in the placenta. The expression of GPCRs was significantly impacted by preeclampsia and the mRNA expression of 22 GPCRs was elevated and 9 GPCRs were down regulated in the preeclamptic placenta as compared to the normal control.

Our in-depth analysis found many GPCRs with remarkable expression profiles, but only a small number of them have been investigated for their function in placental biology¹². Many GPCRs with striking expression profiles in our study have no known role in the placenta and merit additional functional investigation. The Kappa Opioid Receptor (OPRK1), proton-sensing receptor (GPR68) and Atypical Chemokine Receptor 1 (ACKR1) are highly expressed in healthy human placenta markedly downregulated in preeclampsia suggesting a possible role in placental biology. Similarly, uracil nucleotide/cysteiny leukotriene receptor (GPR17), resolvin receptor (GPR32) and Melatonin Receptor 1B (MTNR1B) are significantly upregulated in the preeclamptic placenta and have not been studied before. Insight into the functional significance of these recently discovered GPCRs may help in understanding disease pathophysiology and expedite the drug discovery process.

CONCLUSION

The current study identified the several highly expressed GPCRs with no known role in placental biology, whose expression was altered in preeclamptic human placenta. Understanding the functional significance of these newly identified GPCRs might assist in disease pathogenesis and

offer novel therapeutic opportunities. One of the study's weaknesses is that it used the whole placenta to profile GPCR expression. The placenta is a composite organ and contains many types of cells and as a result, single-cell RNA sequencing will be useful in distinguishing the cell-specific expression of GPCRs in healthy and sick situations. Furthermore, the study is based exclusively on mRNA levels, which may or may not indicate changes in protein levels. As a result, individual GPCR protein levels must be determined using immunoblotting and immunohistochemistry.

SIGNIFICANCE STATEMENT

The expression and function of the vast majority of GPCRs in placental biology are unknown. Re-analysis of RNA sequencing data was performed to examine the mRNA expression of the whole collection of non-visual GPCRs. This study found that the expression of numerous GPCRs was considerably altered in the preeclamptic placenta. The GPCRs with altered expression in diseased placentas may have significant physiological importance in the placenta and could lead to novel treatment approaches.

ACKNOWLEDGMENTS

The authors acknowledge the support for the use of GREIN and TBtools software for data analysis and data extraction. The authors also acknowledge King Abdullah International Medical Research Center (KAIMRC) for supporting this study through research grant RC16/158/R awarded to SM.

REFERENCES

1. Brown, M.A., L.A. Magee, L.C. Kenny, S.A. Karumanchi and F.P. McCarthy *et al.*, 2018. Hypertensive disorders of pregnancy: ISSHP classification, diagnosis, and management recommendations for international practice. *Hypertension*, 72: 24-43.
2. Chappell, L.C., C.A. Cluver, J. Kingdom and S. Tong, 2021. Pre-eclampsia. *Lancet*, 398: 341-354.
3. Burton, G.J., C.W. Redman, J.M. Roberts and A. Moffett, 2019. Pre-eclampsia: Pathophysiology and clinical implications. *BMJ*, Vol. 366. 10.1136/bmj.l2381.
4. Say, L., D. Chou, A. Gemmill, Ö. Tunçalp and A.B. Moller *et al.*, 2014. Global causes of maternal death: A WHO systematic analysis. *Lancet Global Health*, 2: e323-e333.
5. Abalos, E., C. Cuesta, A.L. Grosso, D. Chou and L. Say, 2013. Global and regional estimates of preeclampsia and eclampsia: A systematic review. *Eur. J. Obstet. Gynecol. Reprod. Biol.*, 170: 1-7.

6. Phipps, E.A., R. Thadhani, T. Benzing and S.A. Karumanchi, 2019. Pre-eclampsia: Pathogenesis, novel diagnostics and therapies. *Nat. Rev. Nephrol.*, 15: 275-289.
7. Rana, S., E. Lemoine, J.P. Granger, S.A. Karumanchi, 2019. Preeclampsia: Pathophysiology, challenges, and perspectives. *Circ. Res.*, 124: 1094-1112.
8. Pierce, K.L., R.T. Premont and R.J. Lefkowitz, 2002. Seven-transmembrane receptors. *Nat. Rev. Mol. Cell Biol.*, 3: 639-650.
9. Sriram, K. and P.A. Insel, 2018. G protein-coupled receptors as targets for approved drugs: How many targets and how many drugs? *Mol. Pharmacol.*, 93: 251-258.
10. de Francesco, E.M., F. Sotgia, R.B. Clarke, M.P. Lisanti and M. Maggiolini, 2017. G protein-coupled receptors at the crossroad between physiologic and pathologic angiogenesis: Old paradigms and emerging concepts. *Int. J. Mol. Sci.*, Vol. 18. 10.3390/ijms18122713.
11. West, C. and A.C. Hanyaloglu, 2015. Minireview: Spatial programming of G protein-coupled receptor activity: Decoding signaling in health and disease. *Mol. Endocrinol.*, 29: 1095-1106.
12. Yin, A., X. Guan, J.V. Zhang and J. Niu, 2022. Focusing on the role of secretin/adhesion (Class B) G protein-coupled receptors in placental development and preeclampsia. *Front. Cell Dev. Biol.*, Vol. 10. 10.3389/fcell.2022.959239.
13. Makino, Y., K. Shibata, I. Makino, K. Kangawa and T. Kawarabayashi, 2001. Alteration of the adrenomedullin receptor components gene expression associated with the blood pressure in pregnancy-induced hypertension. *J. Clin. Endocrinol. Metab.*, 86: 5079-5082.
14. Karteris, E., A. Goumenou, E. Koumantakis, E.W. Hillhouse and D.K. Grammatopoulos, 2003. Reduced expression of corticotropin-releasing hormone receptor type-1 α in human preeclamptic and growth-restricted placentas. *J. Clin. Endocrinol. Metab.*, 88: 363-370.
15. Younes, S.T., K.J. Maeda, J. Sasser and M.J. Ryan, 2020. The glucagon-like peptide 1 receptor agonist liraglutide attenuates placental ischemia-induced hypertension. *Am. J. Physiol. Heart Circ. Physiol.*, 318: H72-H77.
16. Buckberry, S., T. Bianco-Miotto, S.J. Bent, V. Clifton, C. Shoubridge, K. Shankar and C.T. Roberts, 2017. Placental transcriptome co-expression analysis reveals conserved regulatory programs across gestation. *BMC Genomics*, Vol. 18. 10.1186/s12864-016-3384-9.
17. Majewska, M., A. Lipka, L. Pauksztó, J.P. Jastrzebski and K. Szeszko *et al.*, 2019. Placenta transcriptome profiling in intrauterine growth restriction (IUGR). *Int. J. Mol. Sci.*, Vol. 20. 10.3390/ijms20061510.
18. Yang, X., J. Yang, X. Liang, Q. Chen and S. Jiang *et al.*, 2020. Landscape of dysregulated placental RNA editing associated with preeclampsia. *Hypertension*, 75: 1532-1541.
19. Al Mahi, N., M.F. Najafabadi, M. Pilarczyk, M. Kouril and M. Medvedovic, 2019. GREIN: An interactive web platform for re-analyzing GEO RNA-seq data. *Sci. Rep.*, Vol. 9. 10.1038/s41598-019-43935-8.
20. Alexander, S.P.H., A. Christopoulos, A.P. Davenport, E. Kelly and A. Mathie *et al.*, 2021. The concise guide to pharmacology 2021/22: G protein-coupled receptors. *Br. J. Pharmacol.*, 178: S27-S156.
21. Chen, C., H. Chen, Y. Zhang, H.R. Thomas, M.H. Frank, Y. He and R. Xia, 2020. TBtools: An integrative toolkit developed for interactive analyses of big biological data. *Mol. Plant*, 13: 1194-1202.
22. Hod, T., A.S. Cerdeira and S.A. Karumanchi, 2015. Molecular mechanisms of preeclampsia. *Cold Spring Harbor Perspect. Med.*, Vol. 5. 10.1101/cshperspect.a023473.
23. Huppertz, B., 2020. Molecular and cellular mechanisms of preeclampsia. *Int. J. Mol. Sci.*, Vol. 21. 10.3390/ijms21134801.
24. Conrad, K.P., 2016. G-protein-coupled receptors as potential drug candidates in preeclampsia: Targeting the relaxin/insulin-like family peptide receptor 1 for treatment and prevention. *Hum. Reprod. Update*, 22: 647-664.
25. Vidal, Jr. M.S., C.D.T. Deguit, G.F.B. Yu and M.D. Amosco, 2022. G-protein coupled receptor dysregulation may play roles in severe preeclampsia-A weighted gene correlation network analysis of placental gene expression profile. *Cells*, Vol. 11. 10.3390/cells11050763.