ORIGINAL ARTICLE

Intrauterine Programming of Adult Disease: Identification of Cardinal Genes Associated with Hypertension, Obesity and Metabolic Syndrome

Programación intrauterina de enfermedad en la vida adulta: identificación de genes cardinales asociados a hipertensión arterial, obesidad y síndrome metabólico

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ABSTRACT

Background: Intrauterine growth restriction is an abnormal fetal development characterized by a fetal growth rate lower than the potential genetic growth for the gestational age. This condition represents a major burden for public health systems, as it increases short and long-term morbidity and mortality in the offspring, particularly because of its association with the development of cardiovascular and metabolic disease in adult life.
Objectives: The aim of the present study was to identify possible cardinal genes involved in intrauterine growth restriction associated with the development of obesity, hypertension and metabolic syndrome using bioinformatics tools.
Methods: A total of 343 genes involved in the phenotypes of interest were obtained and 20 genes were identified as significantly relevant in the interaction network analysis. Specifically, four of these identified genes encode for growth factors or their receptors, VEGFA, PDGFRB, IGF1R and EGFR. We also identified genes related to insulin and cardiovascular homeostasis as CTNNB1, APP, MYC and MDMD2. Cluster analysis provided the most significant gene ontology terms, including those related to the biological processes of proliferation and programmed cell death, intercellular communication, protein metabolism and development of the cardiovascular system.
Conclusions: The genes found in this study could be useful as putative biomarkers for the presence of cardiovascular and metabolic disorders associated with intrauterine growth restriction, or as potential therapeutic targets for treatment strategies directed to the patient's genotype.

Key words: Computational Biology - Gene Ontology - Fetal Growth Retardation - Hypertension - Obesity - Metabolic Syndrome

RESUMEN

Introducción: La restricción del crecimiento intrauterino es una alteración del desarrollo fetal que se caracteriza por una tasa de crecimiento durante la etapa fetal que es menor al potencial genético de crecimiento para la edad gestacional. Esta condición plantea una carga importante para la salud pública, ya que aumenta la morbimortalidad de la descendencia, a corto y a largo plazo, particularmente, por asociarse al desarrollo de enfermedad cardiovascular y metabólica en la vida adulta.
Objetivos: Mediante el uso de herramientas bioinformáticas nos propusimos identificar posibles genes cardinales involucrados en la restricción del crecimiento intrauterino asociados al desarrollo de obesidad, hipertensión arterial y síndrome metabólico.
Material y métodos: Obtuvimos un total de 343 genes involucrados en los fenotipos de interés e identificamos 20 genes que resultaron significativamente relevantes en el análisis de la red de interacción. Particularmente, cuatro de estos genes identificados codifican para factores de crecimiento o sus receptores, VEGFA, PDGFRB, IGF1R y EGFR. Además, identificamos genes relacionados con la insulina y el control de la homeostasis cardiovascular, como son el CTNNB1, APP, MYC y MDMD2. Por otra parte, el análisis de clústeres permitió reconocer los términos de ontología genética más significativos, entre los que se destacan aquellos relacionados con procesos biológicos de proliferación y muerte celular programada, de comunicación intercelular, del metabolismo proteico, y de desarrollo del sistema cardiovascular.
Conclusiones: Los genes hallados en este estudio podrían ser de utilidad como biomarcadores putativos de la presencia de alteraciones cardiovasculares y metabólicas asociadas a la restricción del crecimiento intrauterino o potenciales blancos terapéuticos de estrategias de tratamiento orientadas al genotipo del paciente.

Palabras claves: Biología computacional - Ontología de genes - Retardo del crecimiento fetal - Hipertensión - Obesidad - Síndrome metabólico

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Abbreviations

HPA	Human Protein Atlas	**IUGR**	Intrauterine growth restriction
HTN	Hypertension	**MS**	Metabolic syndrome

INTRODUCTION

Prenatal development is a highly dynamic process involving a combination of maternal, placental, and fetal factors, together with genetic and environmental factors, which can modulate cell proliferation and maturation during the embryonic and fetal stages of intrauterine growth. (1, 2)

Intrauterine growth restriction (IUGR) is an abnormal fetal development that affects 5% to 15% of all live births, (3, 4) and is characterized by a fetal growth rate lower than the potential genetic growth for the gestational age. (2) This complication during pregnancy represents a major burden for health systems, as it increases short and long-term morbidity and mortality in the offspring. (5, 6)

In the eighties, Barker suggested that cardiovascular disease in adults could originate in fetal life based on the observation of the geographic distribution of infant mortality at the beginning of the twentieth century, which was similar to mortality due to ischemic heart disease approximately 60 years later. (7) Since then, several epidemiological, clinical, and experimental studies have been carried out, which made it possible to further understand the factors that can negatively affect fetal development, including maternal malnutrition, gestational hypertension, preeclampsia/eclampsia, gestational diabetes, reduced renin angiotensin system activity in the kidneys, and exposure to hypoxia or stress. (2) Particularly, many studies have supported the hypothesis of early programming of cardiovascular disease in adult life, nutrient supply to the fetus being one of the main causes. (8-11) Furthermore, to preserve energy and survive at the expense of completing fetal growth in an environment of inadequate nutrient supply, impaired fetal glucose and insulin homeostasis have been observed, modifying the metabolic profile in tissues and programming the expression of adaptations in postnatal life associated with insulin resistance and excess weight. (12) Moreover, exposure to damage during intrauterine development can have metabolic consequences in later generations. (13, 14)

In modern society, including Argentina, there is a high prevalence of risk factors for the development of cardiovascular and metabolic diseases. (15) Therefore, the development of strategies for the early detection of the propensity of an individual born with IUGR to develop cardiovascular or metabolic disease in adult life could mitigate the risk of long-term consequences of IUGR through targeted therapeutic interventions and treatment strategies.

Nowadays, there are considerable data in the literature about IUGR, hypertension (HTN), obesity and metabolic syndrome (MS). The latter represents the association of many cardiovascular risk factors as obesity, HTN, dyslipidemia, and insulin resistance. (16) However, in the literature these data are divided into different levels of biological information (genomics, transcriptomics and proteomics). Processing and integrating data using bioinformatics tools facilitates the application of personalized medicine.

Thus, the aim of the present study was to evaluate and identify the possible cardinal genes involved in IUGR associated with the development of HTN, obesity and MS, and the potentially abnormal biological processes.

METHODS

Data collection

The genes related to IUGR, obesity, HTN and MS were collected in July 2019 from the following databases: Phenotype-Genotype Integrator (PheGenI https://www.ncbi.nlm.nih.gov/gap/phegeni), Gene NCBI (https://www.ncbi.nlm.nih.gov/gene), Clinvar (http://www.ncbi.nlm.nih.gov/clinvar/), (17) Database of gene-disease associations (DisGeNET https://www.disgenet.org/), (18) European Bioinformatic Institute (EBI http://www.ebi.ac.uk) (19) and Comparative Toxicogenomics Database (CTD http://ctdbase.org) (20), in the latter case selecting only genes with a direct relationship to phenotypes based on the published literature.

Construction of genetic interaction networks

Genetic interaction networks are a mathematical representation of the interaction between protein-coding genes in biological systems. This relationship is established through different methods, such as text mining, information based on experiments, interactions established by orthology models, statistical methods, and predictions based on protein characteristics. The interaction networks were constructed using three widely used tools: Search Tool for the Retrieval of Interaction Genes/Protein (STRING https://string-db.org/) (21) created by the Swiss Institute of Bioinformatics (SIB) and the European Molecular Biology Laboratory (EMBL); Consensus Path DB-Human (CPDB http://cpdb.molgen.mpg.de/), (22) developed by the MAX Planck Institute for Molecular Genetics; and Gene MANIA (https://genemania.org/) (23) developed by the Donnelly Centre for Cellular and Biomolecular Research; University of Toronto, Canada.

Analysis of genetic interaction networks and topological evaluation

The networks were visualized and analyzed with Cytoscape V3 software. (24) The most relevant genes were determined in relation to degree of centrality, a measure of topological centrality that defines the number of edges incident upon a node: i.e., degree centrality is used to identify the relevant genes directly involved with other protein-coding genes in the network. The following equation is used:

For a graph B and a node υi, deg represents the number of edges.

$$B(vi) = deg(vi)$$

Betweenness centrality is another measure considered and represents the number of times a node acts as a bridge between other nodes. Thus, this measure shows key connectors for the dynamics of a biological network. Betweenness centrality is calculated using the following formula: (26)

For a graph B, a node υi and two nodes different from υ (s and t), σst is the total number of shortest paths from node s to node t and σst (υi) is the number of those paths that pass through υi.

$$B(v_i) = \sum_{s \neq v_i \neq t} \frac{\sigma_{st}(v_i)}{\sigma_{st}}$$

Besides the estimation of centrality measures, we investigated, for descriptive purposes, the tissue profiles in which these genes were overexpressed. For this end, we used the Human Protein Atlas (HPA), (27) a platform developed by the Karolinska Institute in association with other institutions, which provides information of transcript expression from different sources. Moreover, additional packages of Cytoscape were installed to detect densely connected regions or protein clusters, using the Molecular Complex Detection (MCODE) algorithm (28) with a Degree of 3, a Node score of 0.2 and a K-score of 2.

Functional analysis

Functional enrichment analysis of genes was carried out using g.Profiler (https://biit.cs.ut.ee/gprofiler/gost). (29) This public web server provides a collection of tools used in a standardized way in biological analyses, allowing the identification of biological pathways and gene ontology categories including biological processes (BP), molecular functions (MF) and cellular components (CC). The Benjamini-Hochberg procedure was used for calculating the false discovery rate (FDR). A p value <0.01 and FDR <0.05 were considered statistically significant. Additionally, automated electronic annotations were excluded and at least 5 query terms were required to accept a functional annotation to reduce the probability of false positives. Next, the most significant gene ontology terms meeting the FDR criterion were selected and redundant terms were removed using the REVIGO tool (Reduce and Visualize Gene Ontology, http://revigo.irb.hr/). (30)

Ethical considerations

Not applicable.

RESULTS

Gene list

A total of 5,605, 759, 2,787 and 532 genes related to the phenotypes of obesity, HTN, MS and IUGR, respectively, were obtained. Using the set theory, 343 overlapping genes were detected between IUGR and the other three disorders (Figure 1).

Genetic interaction network

The 343 overlapping genes were used to create the networks. The network obtained contained 12,633 connections (Figure 2).

Next, the top 20 genes were obtained: EGFR, epidermal growth factor receptor; MAPK1, mitogen-activated protein kinase 1; STAT3, signal transducer and activator of transcription 3; CTNNB1, catenin beta 1;

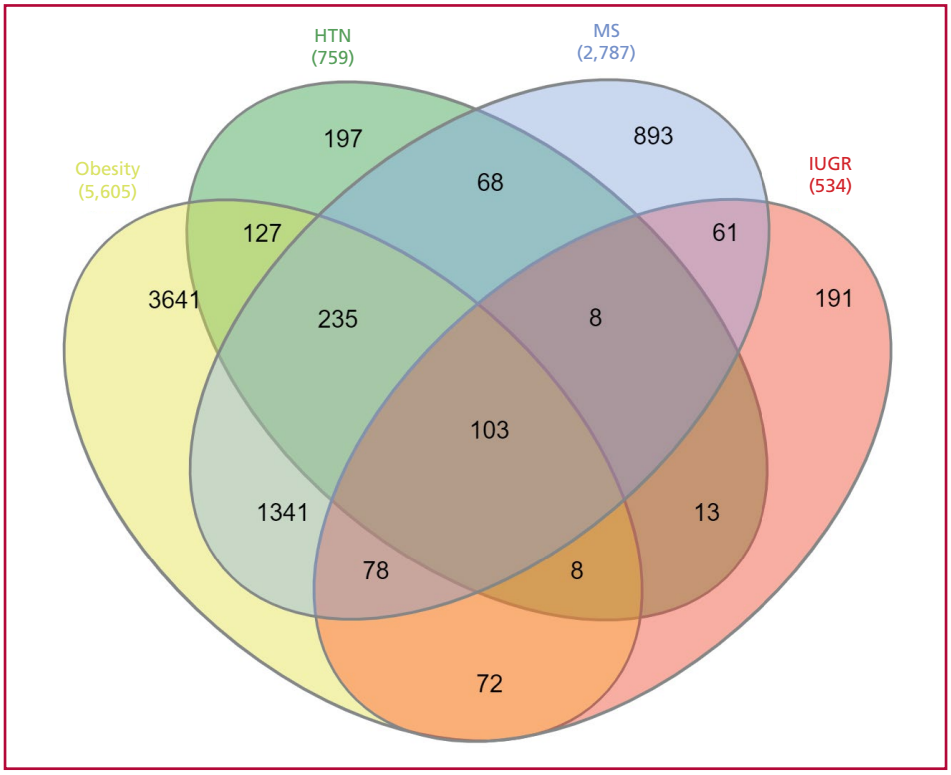


Fig. 1. Venn diagram of genes with abnormal expression in the phenotypes of obesity, hypertension, metabolic syndrome, and intrauterine growth restriction

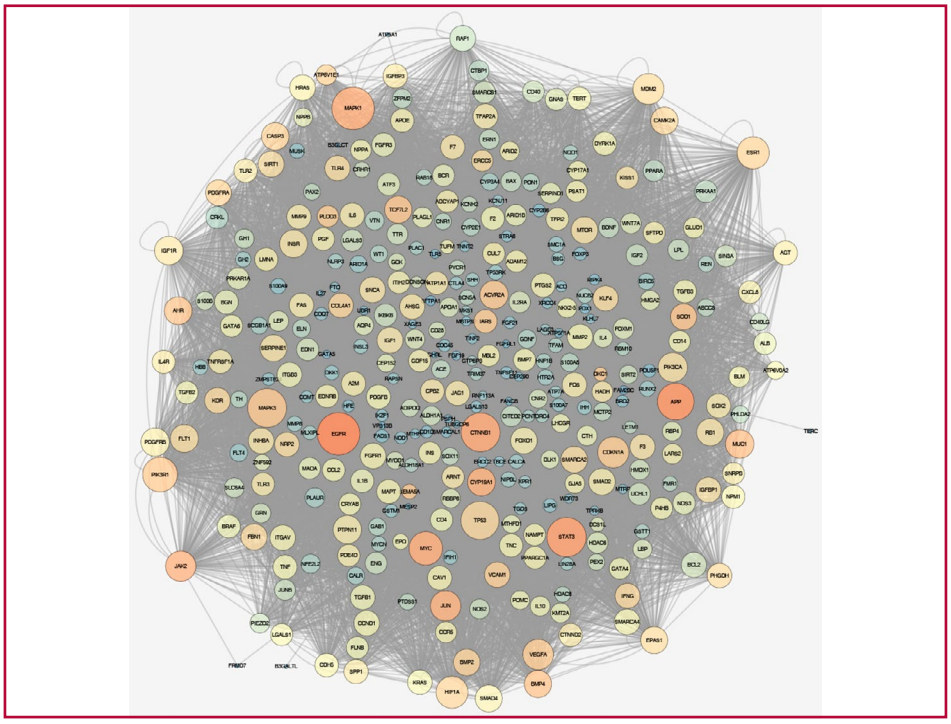


Fig. 2. Unified genetic integration network of 343 nodes with 12,633 interactions. The node diameter is directly related to degree centrality and the color to betweenness, where the closer to red color, the higher the value for that gene

MAPK3, mitogen-activated protein kinase 3; TP53, tumor suppressor protein 53; APP, amyloid precursor protein; PIK3R1, phosphoinositide-3-kinase regulatory subunit 1; MYC proto-oncogene bHLHe39, basic helix-loop-helix protein 39; ESR1, estrogen receptor-alpha; HIF1A, hypoxia-inducible factor 1-alpha; CDKN1A, cyclin-dependent kinase inhibitor 1A; MDM2, MDM2proto-oncogene; VEGFA, vascular endothelial growth factor A; JAK2, janus kinase 2; JUN, c-jun, AP-1 transcription factor subunit; PIK3CA, phosphatidylinositol 3-kinase catalytic subunit alpha; IGF1R, insulin-like growth factor 1 receptor; MUC1, mucin 1, cell surface associated; and PDGFRB, platelet derived growth factor receptor beta. The results of the topological analysis revealed that degree centrality ranged from 128 to 239 and betweenness centrality from 0.00342065 to 0.0131197 (Table 1). Gene ontology terms, main gene function and overexpression in tissues are shown in Table 1. Some genes have ubiquitous expression (Table 1).

Protein clusters

After identifying the most important protein-coding genes in the network, further analysis was performed on the network. As previously mentioned, the network is made up of genes overlapping between IUGR and conditions representing cardiovascular risk such as obesity, HTN and MS. Therefore, we proceeded to locate dense areas of protein complexes and then continued with the identification of ontology terms involved in each of the clusters found (Figure 3). Clusters with less than 3 nodes were excluded because they could show unreliable results in the determination of ontology terms. As result, eight clusters were identified, made up of 5 to 50 nodes (Figure 3)

DISCUSSION

To our knowledge, this study is the first to use network analysis to evaluate the complex interaction between IUGR and the phenotypes of obesity, HTN, and MS, in search of cardinal genes that could potentially function as putative biomarkers of the development of these phenotypes in individuals who presented with IUGR or that could be used as potential therapeutic targets.

We identified a total of 20 genes that were significantly relevant in the analysis of the interaction network; most of these genes were related to intracellular signaling pathways associated with response to organic substances and positive regulation of cellular biosynthesis. According to the results obtained, at least four of the genes identified encode for growth factors or their receptors: VEGFA, PDGFRB, EGFR and IGF1R. The identification of IGF1R stands out, as its ligand, insulin-like growth factor 1 (IGF1), synthesized in the liver, though ubiquitously expressed, can modulate carbohydrate and lipid metabolism. The release of IGF1 is stimulated by the growth hormone and insulin. When caloric restriction is present, IGF1 levels decrease and its synthesis in the liver is refractory to growth hormone stimulation. (31) This process limits growth and protein synthesis when nutrient availability is compromised. In pathophysiological states, including increased insulin resistance, the number of IGF1R changes significantly, potentially abrogating the chance of IGF1 to alter glucose metabolism, and is

Table 1. Top 20 central genes of the interaction network Genes with ubiquitous distribution are identified with*

Gene	Degree ofcentrality	Betweenness centrality	Protein	Locus	Overexpression in tissue	GO terms	Overexpression in tissue
EGFR	239	0.0131197	Epidermal growth factor receptor	7p11.2	Transmembrane glycoprotein. Positive regulation of cell proliferation and migration.	Cellular response to organic substance, positive regulation of cellular biosynthesis, regulation of cell proliferation, positive regulation of cellular biosynthesis, regulation of phosphatidylinositol 3-kinase signaling, enzyme binding, phosphatase binding, identical protein binding, phosphatase binding, phosphotransferase activity, alcohol group as acceptor, focal adhesion, membrane raft	Placenta, breast, skin, liver, adipose tissue
MAPK1	239	0.00933646	Mitogen-activated protein kinase 1	22q11.22	Depending on the cellular context it mediates diverse biological functions such as cell growth, adhesion, survival and differentiation.	Cellular response to organic substance, positive regulation of cellular biosynthesis, regulation of phosphatidylinositol 3-kinase signaling, enzyme binding, phosphatase binding, identical protein binding, phosphotransferase activity, alcohol group as acceptor, focal adhesion, membrane raft	Basal ganglia, cerebral cortex, amygdala, skeletal muscle
STAT3	214	0.01061652	Signal transducer and activator of transcription 3	17q21.2	Cellular responses to growth factors. Maintains steady state in the β-cells	Cellular response to organic substance, positive regulation of cellular biosynthesis, regulation of cell proliferation, enzyme binding, phosphatase binding, identical protein binding, transcription factor binding, RNA polymerase II transcription factor complex	Liver, cardiac muscle, granulocytes, skeletal muscle, vagina, smooth muscle
CTNNB1	211	0.00990833	Catenin beta 1	3p22.1	Creation and maintenance of epithelial cell layers. Involved in regulation of insulin internalization.	Cellular response to organic substance, positive regulation of cellular biosynthesis, regulation of cell proliferation, positive regulation of cellular biosynthesis, enzyme binding, phosphatase binding, transcription factor binding, focal adhesion, RNA polymerase II transcription factor complex, membrane raft	*Ovary, placenta, endometrium, cerebellum, retina, smooth muscle, thyroid gland
MAPK3	210	0.00675088	Mitogen-activated protein kinase 3	16p11.2	Apoptosis, cell proliferation, adhesion, differentiation, endosomal dynamics and cell survival.	Cellular response to organic substance, positive regulation of cellular biosynthesis, regulation of phosphatidylinositol 3-kinase signaling, enzyme binding, phosphatase binding, identical protein binding, focal adhesion, phosphotransferase activity, alcohol group as acceptor, membrane raft	*Amygdala, cerebral cortex, small intestine, colon, esophagus
TP53	204	0.00518495	Tumor suppressor protein 53	17p13.1	Transcription factor with antitumoral activity through negative regulation of genes involved in cell proliferation.	Cellular response to organic substance, positive regulation of cellular biosynthesis, regulation of cell proliferation, enzyme binding, phosphatase binding, identical protein binding, transcription factor binding, p53 binding, RNA polymerase II transcription factor complex	Thymus, ovary, appendix, lymph nodes, esophagus, skin
APP	193	0.01175245	Amyloid precursor protein	21q21.3	Cell surface receptor and transmembrane precursor. Involved in cell motility and transcription regulation. Related to internalization of amyloid-beta peptide leading to mitochondrial dysfunction.	Cellular response to organic substance, positive regulation of cellular biosynthesis, regulation of cell proliferation, cellular response to organic substance, enzyme binding, identical protein binding, membrane raft	*Nervous system, kidney, adrenal gland, breast tissue, placenta, smooth muscle, heart muscle
PIK3R1	179	0.00742799	Phosphoinositide-3-kinase regulatory subunit 1	5q13.1	Insulin metabolism. Modulates cellular response to endoplasmic reticulum stress.	Cellular response to organic substance, positive regulation of cellular biosynthesis, regulation of phosphatidylinositol 3-kinase signaling, enzyme binding, phosphatase binding, transcription factor binding, RNA polymerase II transcription factor complex	*Salivary gland, bone marrow, skeletal muscle, liver, breast tissue, cerebral cortex, smooth muscle, heart muscle

(continue)

(continuation)

Gene	Degree ofcentrality	Betweenness centrality	Protein	Locus	Overexpression in tissue	GO terms	Overexpression in tissue
MYC	168	0.00936675	Proto-oncogene bHLHe39, basic helix-loop-helix protein 39	8q24.21	Cell-cycle progression and differentiation. Promotes angiogenesis. Regulator of somatic reprogramming, as it controls self-renewal of embryonic stem cells.	Cellular response to organic substance, positive regulation of cellular biosynthesis, regulation of cell proliferation, transcription factor binding, RNA polymerase II transcription factor complex	*Skin, skeletal muscle, adipose tissue, pancreas, Fallopian tube, T-cells, lymphoid tissue
ESR1	158	0.00630876	Estrogen receptor-alpha	6q25.1-q25.2	Regulates cellular processes including growth, differentiation and function of the reproductive system. Maintenance of skeletal cardiovascular and nervous system homeostasis.	Cellular response to organic substance, positive regulation of cellular biosynthesis, enzyme binding, phosphatase binding, identical protein binding, transcription factor binding, RNA polymerase II transcription factor complex	Endometrium, cervix, epididymis, smooth muscle, breast tissue
HIF1A	157	0.00598343	Hypoxia-inducible factor 1 subunit alpha	14q23.2	Master regulator of hypoxic response. Embryonic vascularization, angiogenesis and pathophysiology of ischemic disease.	Cellular response to organic substance, positive regulation of cellular biosynthesis, regulation of cell proliferation, enzyme binding, phosphatase binding, transcription factor binding, p53 binding, RNA polymerase II transcription factor complex	*Bone marrow, kidney, adrenal gland, vessel, granulocytes, cervix, lungs
CDKN1A	150	0.00635673	Cyclin-dependent kinase inhibitor 1A	6p21.2	Negative regulation of cell cycle progression and response to DNA damage.	Cellular response to organic substance, positive regulation of cellular biosynthesis, regulation of cell proliferation, enzyme binding, phosphatase binding, phosphotransferase activity, alcohol group as acceptor, RNA polymerase II transcription factor complex	*Small intestine, liver, skeletal muscle, adipose tissue, esophagus, colon
MDM2	149	0.00535669	MDM2 proto-oncogene	12q15	Mediates ubiquitination and degradation of p53/TP53, promoting tumor development. Negative regulation of AMPA receptors, necessary for baroreceptor reflex function in response to blood pressure changes.	Cellular response to organic substance, positive regulation of cellular biosynthesis, regulation of cell proliferation, enzyme binding, identical protein binding, phosphatase binding, p53 binding	*Thymus, liver, placenta, bone marrow, pancreas, skeletal muscle, granulocytes
VEGFA	145	0.00646737	Vascular endothelial growth factor A	6p21.1	Angiogenesis, vasculogenesis and endothelial cell growth. Related to microvascular complications of diabetes.	Cellular response to organic substance, positive regulation of cellular biosynthesis, regulation of cell proliferation, identical protein binding, focal adhesion	*Thyroid gland, liver, heart muscle, endometrium, skeletal muscle, urinary bladder, prostate
JAK2	143	0.01015	Janus kinase 2	9p24.1	Signaling events in innate and adaptive immunity. In the cytoplasm it mediates signal transduction of hormones as leptin, prolactin, erythropoietin and thrombopoietin. Phosphorylates STAT.	Cellular response to organic substance, positive regulation of cellular biosynthesis, regulation of cell proliferation, regulation of phosphatidylinositol 3-kinase signaling, enzyme binding, phosphatase binding, identical protein binding, phosphotransferase activity, alcohol group as acceptor, focal adhesion, membrane raft	Granulocytes, cardiac muscle, vessel, lymph nodes, skin
JUN	143	0.00874665	C-jun, AP-1 transcription factor subunit	1p32.1	Transcription factor that increases steroidogenic gene expression. Involved in malignant tumors and optic nerve hypertensive injury.	Cellular response to organic substance, positive regulation of cellular biosynthesis, regulation of cell proliferation, enzyme binding, identical protein binding, transcription factor binding, phosphatase binding, RNA polymerase II transcription factor complex	*Female reproductive system, bone marrow, adipose tissue, thyroid gland, skin, lung
PIK3CA	136	0.00425497	Phosphoinositol-3-kinase catalytic subunit 1 alpha	3q26.32	Participates in cellular signaling in response to growth factors as insulin, VEGFA or PDGF. Participates in cardiomyogenesis in embryonic stem cells through a AKT1 pathway.	Cellular response to organic substance, regulation of phosphatidylinositol 3-kinase signaling, phosphotransferase activity, alcohol group as acceptor, RNA polymerase II transcription factor complex	*Thymus, bone marrow, parathyroid gland, cerebral cortex, breast, adipose tissue

(continue)

(continuation)

Gene	Degree ofcentrality	Betweenness centrality	Protein	Locus	Overexpression in tissue	GO terms	Overexpression in tissue
IGF1R	130	0.00415681	Insulin-like growth factor 1 receptor	15q26.3	Survival of malignant cells. Activation of PI3K-AKT/PKB and Ras-MAPK signaling pathways, inhibiting apoptosis and increasing cellular proliferation.	Cellular response to organic substance, regulation of cell proliferation, regulation of phosphatidylinositol 3-kinase signaling, identical protein binding, phosphotransferase activity, alcohol group as acceptor, RNA polymerase II transcription factor complex	*Seminal vesicle, ovary, cerebellum, pancreas, prostate, smooth muscle, placenta
MUC1	129	0.00825035	Epithelial mucin 1	1q22	Plays a role in forming protective mucous barriers on epithelial surfaces. Intracellular signaling of ERK, SRC, NF-kappa-B and Ras/MAPK pathways.	Cellular response to organic substance, positive regulation of cellular biosynthesis, p53 binding, membrane raft, RNA polymerase II transcription factor complex	Gallbladder, kidney, lung, stomach, pancreas, esophagus
PDGFRB	128	0.00342065	Platelet derived growth factor receptor beta	5q32	Mitogen for cells of mesenchymal origin. Essential for normal development of the cardiovascular system. Involved in internalization of receptors, angiogenesis, migration of vascular smooth muscle cells and rearrangement of the actin cytoskeleton	Cellular response to organic substance, positive regulation of cellular biosynthesis, regulation of cell proliferation, regulation of phosphatidylinositol 3-kinase signaling, enzyme binding, phosphatase binding, phosphotransferase activity, alcohol group as acceptor, focal adhesion, membrane raft	Female reproductive system, adipose tissue, smooth muscle, skeletal muscle, cardiac muscle

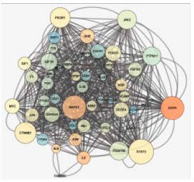
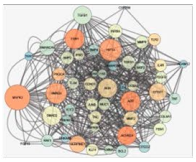
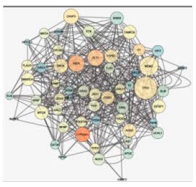
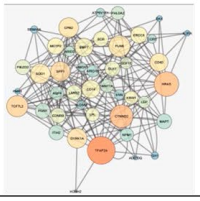
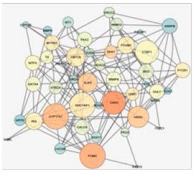
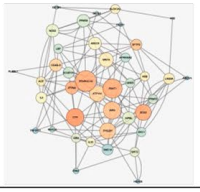
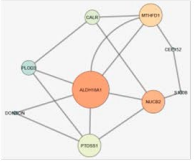
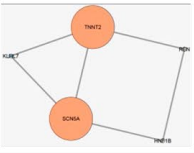
Clúster	Genes	Términos de ontología genética	MCODE *Score*
1 	*JAK2, PIK3R1, PTPN11, EGFR, STAT3, PDGFRB, GALS13, SIRT1, AHR, SERPIN, FGFR1, FOXO1, CYP2E1, IGF1, F3, IGF1R, MBL2, MLXPL, RBP4, FOS, MYC, JUN, CTNNB1, CDKN1A, CAV1, PTGS2, CDKN1A, ITGAV, MAPK1, GLUD1, KDR, FGFR3, CCL2, RB1, A2M, ALB, IL6, FAM20C, CXCL8, ARNT, APOA, CYP3A1, PDGFRB, LGALS13, VEGFA, FLT4, VCAM1, PLAC1, GH2*	Positive regulation of cell proliferation; response to chemical components; regulation of apoptotic process; focal adhesion; endomembrane system; binding of growth factors; protein kinase activity	20,082
2 	*MAPK3, FGF19, RAF1, PRKAA1, SERPINE1, SMAD2, AGTF3, SMAD4, PIK3CA, TLR4, TERT, SMARCA4, BMP2, IFNG, ESR1, TGFB1, BMP4, GAB, FAS, CCND1, BGN, MUC1, JUNB, TNC, TNFRSF1A, KLF4, PRKAR1A, LMNA, BCL2, ACVR2A, CITED2, FBN1, COL4A1, TNF, APP, CD4, S100A8, EPAS1, PDGFB, IL4R, ADAM12, NRP2, TGFB3, SMARCB1, HIF1A, INHBA, MMP9, TLR2, IL4R, PDGFB, CYP2B6*	Positive regulation of cell proliferation; response to chemical components; regulation of apoptotic process; focal adhesion; endomembrane system; binding of growth factors; protein kinase activity	18,245
3 	*APOE, NOS3, TFPI2, AGT, AHSG, PDE4D, CRKL, UCHL1, P4HB, IGFBP1, BLM, TP53, MDM2, IGF2, F7, RUNX2, BIRC5, F2, LGALS1, CAMK2A, IKBKB, VTN, CASP3, S100A9, MAOA, NFE2L2, CRYAB, INSR, FLT1, TGFB2, TLR3, SNCA, SIN3A, PLAUR, SMARCA2, ELN, BRAF, MTOR, LGALS3, MUSK, GDNF, NAMPT, NLRP3, BDNF, GATA6, RIPK4, PGF, CYP19A1, PDGFRA, TP53RK, JAG1, KRAS, ITGB3*	Positive regulation of response to stimuli; regulation of protein metabolism; extracellular region; cell-cell junctions; growth factor activity; insulin-like growth factor binding	13,815
4 	*TCF7L2, FMR1, PON1, EDNRB, ITIH2, DYRK1A, LPL, KCNH2, TFAP2A, NPM1, CTNND2, ADIPOQ, GH1, MAPT, LEP, KISS1, ENG, SLC6A4, AQP4, LARS2, CD14, WNT7A, HRAS, CD40, CCR5, IL2RA, BAX, ERCC5, FLNB, GDF15, DLK1, ARID1B, HMOX1, BCR, SPP1, SOD1, PIEZO2, SEMASA, MCTP2, ARID2, BMP7, CPB2, ATP6V1E1, PHLDA2*	Positive regulation of cell communication; response to chemical components; extracellular region; cell-cell junctions; protein homodimerization activity; apolipoprotein binding	9,302
5 	*CEP290, MMP8, WT1, MYOD1, TH. NPPA, GATA4, SMARCAL1, KMT2A, CD28, PAX2, HTR2A, ORC4, GJA5, HDAC8, DKK1, FOXM1, RBBP8, CNR2, NPPB, FOXP3, ADCYAP1, CALCA, CYP17A1, INS, GHRL, SOX11, PEX2, CNR1, HADH, LHCGR, POMC, RAB18, ERCC2, RBM10, PQU5F1, CTBP1, BSG, FTO, CUL7, MYCN, FGF21, PYRCR1, SNRPB*	Cellular biosynthesis; chromosome; nucleus; RNA polymerase II transcription factor activity; double-stranded DNA binding	6,773
6 	*PLAGL1, TNFSF11, NKX2-5, IL4, ACE, CD40LG, CTH, SCGB1A1, ZFPM2, GNR, LBP, NOS2, FPARGC1A, ATP1A1, PSAT1, IARS, IL10, PHGDH, SMC1A, ERN1, WNT4, ABCC8, GSTM1, PPARA, GATA5, ALDH1A1, FADS1, HFE, HDAC6, SIRT2, NIPBL, DKC1, SOX2, GNAS, HBB, TRIM37, LIN28A, ARID1A, ATP6V0A2, SFTPD*	Response to stress; positive metabolic regulation; chromosome; extracellular region; ATPase-coupled cation transmembrane transporter activity; chromatin binding	6,103
7 	*DONSON, PLOD3, PTDSS1, ALDH18A1, NUCB2, CALR, MTHFD1, CEP152, S100B*	Calcium ion binding; opsonin binding	3,5
8 	*KLHL7, SCN5A, HNF1B, TNNT2, REN*	Formation of the left ventricle; actin-mediated cell contraction; circulatory system	3

Fig. 3. Protein clusters and the most significant ontology terms identified. Ontology terms in blue correspond to biological processes, in orange to cellular compartments, and in green to molecular function

therefore associated with the development or perpetuation of MS. (32)

On the other hand, the identification of four cardinal genes that are expressed in tissues closely related to the phenotypes studied, such as the placenta, different types of muscle and adipose tissue is interesting, as they have not yet been evaluated in practice as biomarkers of the phenotypes included in this study.

The CTNNB1 gene is involved in insulin internalization and its protein expression has been found to be a signaling pathway involved in cardiac hypertrophy, (33) in addition to negatively modulate adipocyte differentiation. (34) Thus, this gene may be of interest for the three phenotypes studied.

The identification of the APP gene, which is expressed in the placenta and in cardiac and smooth muscle tissue, according to HPA analysis, is related to cellular transcription processes. The identification is this gene is interesting, as it is commonly associated with Alzheimer's disease, but is currently also associated with MS. The intracellular insulin signaling pathway is involved in the metabolism of amyloid protein, which can bind to insulin receptors triggering their internalization, thus decreasing the response of neurons to insulin and promoting insulin resistance. (35)

In addition, the identification of two proto-oncogenes, MYC and MDMD2 in the analysis of gene interaction networks highlights the relevance of cell-cycle progression and differentiation processes. Both proto-oncogenes involved in maintaining cardiovascular homeostasis have been poorly explored in the literature in relation with these phenotypes, and their inclusion as potential biomarkers or therapeutic targets is also of interest.

Moreover, cluster analysis provided us with the most significant gene ontology terms, which were classified according to the value of FDR or corrected p value, so that significance was higher the lower the value obtained. The most outstanding ontological terms are those related to the biological processes of proliferation and programmed cell death, intercellular communication, protein metabolism and development of the cardiovascular system. These findings are consistent with the evidence showing that these processes are key factors for the development of organ and tissue disorders. (9, 36) We also found that the genes identified encode for proteins that can be found in the extracellular compartment, allowing for their effective use as biomarkers, and their molecular functions are closely related to growth factors and DNA stability.

The main strength of this study was the use of information globally available, by processing and analyzing data from different levels of biological information. We also used methods with highly reliable statistical analysis. This analysis also helps to guide further studies, reducing the time and resources needed for the evaluation of biomarkers and therapeutic targets, and allowing for a more targeted approach.

However, there are certain limitations as population variability in the results obtained, and these findings should be confirmed by experimental evaluation. Nevertheless, the use of six databases to initially obtain the set of genes for each phenotype, together with the use of three different tools to establish the genetic interaction networks, ensures that they complement each other.

In conclusion, the genes found in this study could be useful as putative biomarkers for the presence of cardiovascular and metabolic disorders associated with IUGR or could be useful as potential therapeutic targets of treatment strategies directed to the patient's genotype.

Author contribution criteria

IDAM: Data collection, analysis and interpretation, manuscript drafting; DAS, AT, RE, CA: Data interpretation and manuscript review; CC: Design, data interpretation, manuscript drafting and review

Conflicts of interest

None declared.

(See authors' conflicts of interest forms on the website/ Supplementary material)

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