PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
DOUTORADO EM FISIOPATOLOGIA E SAÚDE ANIMAL

THAOAN BRUNO MARIANO

1 - EFEITO DO TREINAMENTO PREVENTIVO NO TRANSCRIPTOMA CARDÍACO DE RATOS COM HIPERTENSÃO PULMONAR
2 - TRADUÇÃO PARA O PORTUGUES DA FERRAMENTA SYRCLE DE RISCO DE VIÉS

Presidente Prudente - SP
2022

PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
DOUTORADO EM FISIOPATOLOGIA E SAÚDE ANIMAL

THAOAN BRUNO MARIANO

1 - EFEITO DO TREINAMENTO PREVENTIVO NO TRANSCRIPTOMA CARDÍACO DE RATOS COM HIPERTENSÃO PULMONAR
2 - TRADUÇÃO PARA O PORTUGUES DA FERRAMENTA SYRCLE DE RISCO DE VIÉS

Tese de Doutorado apresentada Pró-Reitoria de Pesquisa e Pós-Graduação, Universidade do Oeste Paulista, como parte dos requisitos para obtenção do título de Doutor – Área de concentração: Fisiopatologia e Saúde Animal

Orientadora:
Profª. Drª. Francis Lopes Pacagnelli

Presidente Prudente - SP
2022

616.12
M333e

Mariano, Thaoan Bruno.
1- Efeito do treinamento preventivo no transcriptoma cardíaco de ratos com hipertensão pulmonar. 2- Tradução para o português da ferramenta syrcle de risco de viés / Thaoan Bruno Mariano. – Presidente Prudente, 2022.
105f.: il.

Dissertação (Doutorado em Fisiopatologia e Saúde Animal) - Universidade do Oeste Paulista – Unoeste, Presidente Prudente, SP, 2022.
Bibliografia.
Orientador: Francis Lopes Pacagnelli.

1. Disfunção cardíaca. 2. Hipertrofia cardíaca. 3. Hipertensão arterial pulmonary. I. Título.

Catalogação na fonte: Michele Mologni – CRB 8 - 6204

THAOAN BRUNO MARIANO

1 - EFEITO DO TREINAMENTO PREVENTIVO NO TRANSCRIPTOMA CARDÍACO DE RATOS COM HIPERTENSÃO PULMONAR
2 - TRADUÇÃO PARA O PORTUGUES DA FERRAMENTA SYRCLE DE RISCO DE VIÉS

Tese de Doutorado apresentada Pró-Reitoria de Pesquisa e Pós-Graduação, Universidade do Oeste Paulista, como parte dos requisitos para obtenção do título de Doutor - Área de Concentração: Fisiopatologia e Saúde Animal.

Presidente Prudente, 20 de Junho de 2022.

BANCA EXAMINADORA

_____
Prof. Dra. Francis Lopes Pacagnelli
Universidade do Oeste Paulista – Unoeste
Presidente Prudente-SP

_____
Prof. Dra. Mariana Janini Gomes
Research Fellow, Brigham and Women's Hospital/ Harvard Medical School
Boston - EUA

_____
Prof. Dr. Robson Chacon Castoldi
Universidade Federal de Mato Grosso do Sul – UFMS
Campo Grande -MS

_____
Prof. Dra. Inês Cristina Giometti
Universidade do Oeste Paulista – Unoeste
Presidente Prudente-SP

_____
Prof. Dr. Edson Assunção Mareco
Universidade do Oeste Paulista – Unoeste
Presidente Prudente-SP

DEDICATÓRIA

A minha esposa Larissa Ferreira Rós Mariano, que transformou tudo desde que surgiu. Espero conseguir te dar de volta pelo menos uma gota do oceano de felicidade que você me trouxe desde que chegou na minha vida.

Aos meus pais Martim e Cristina, e minha irmã Annelise por todo carinho, apoio e ajuda que me deram por toda minha vida.

À minha orientadora Profa. Dra. Francis Lopes Pacagnelli, por todo conhecimento e incentivo desde a graduação, pelas oportunidades ofertadas de crescimento profissional, por todo carinho e dedicação.

AGRADECIMENTOS

Primeiramente, ao Criador que me abençoou e proporcionou conhecimento para esta jornada.

À minha família que sempre me apoiou e acreditou na minha capacidade.

À minha orientadora Profa. Dra. Francis Lopes Pacagnelli, que proporcionou esta oportunidade profissional e sempre me incentivou a explorar o máximo da minha capacidade. Sou muito grato pela oportunidade. Obrigado!

Aos Docentes Prof. Dr. Edson Assunção Mareco, Prof. Dr. Robson Francisco Carvalho, Prof. Dr. Katashi Okoshi, Prof. Dr. Antônio Carlos Cicogna, e Prof. Dr. Luis Antonio Justulin Junior que colaboraram para a realização desse trabalho.

Aos colegas Me. Igor de Carvalho Deprá**,** Dr. Dijon pelo apoio, Dra. Paula Paccieli Freire, Dra. Sarah Santiloni Cury, Dra. Danielle Fernandes Vileigas conhecimento e por toda ajuda na parte experimental deste trabalho.

Aos colegas do Doutorado em Fisiopatologia e Saúde Animal e profissionais da UNOESTE de Presidente Prudente e da UNESP de Botucatu, que ajudaram de alguma forma.

Aos Docentes do Doutorado que compartilharam todo conhecimento que possuem, contribuindo com a minha formação.

À UNOESTE, pela oportunidade do Doutorado, bem como toda a sua equipe de profissionais, que tornam esta universidade um lugar de respeito e exemplo a ser seguido.

À Faculdade de Medicina da UNESP de Botucatu, que deu todo apoio científico e tecnológico para a realização deste Doutorado.

À Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior- CAPES, pela bolsa concedida, que usufrui no primeiro semestre de 2018.
“O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – (Brasil) CAPES – Código de Financiamento 001”.

O presente trabalho foi realizado com apoio da Fundação de Amparo a pesquisa do Estado de São Paulo (Fapesp) processo 2016/ 11344-0.

A todos os meus sinceros agradecimentos!

“Os homens perdem a saúde para juntar dinheiro, depois perdem o dinheiro para recuperar a saúde. E por pensarem ansiosamente no futuro esquecem do presente de forma que acabam por não viver nem no presente nem no futuro. E vivem como se nunca fossem morrer... e morrem como se nunca tivessem vivido.”

Dalai Lama

RESUMO

1 - Efeito do treinamento preventivo no transcriptoma cardíaco de ratos com hipertensão pulmonar
2 - Tradução para o português da ferramenta syrcle de risco de viés

Introdução 1: A indução da hipertensão pulmonar (HP) por monocrotalina é comum, o que leva a pós-carga ventricular direita elevada, remodelação vascular, hipertrofia e disfunção ventricular direita e, por fim, insuficiência cardíaca. O exercício físico tem sido reconhecido como um tratamento para esta condição. Embora diferentes mecanismos tenham sido propostos para explicar o desenvolvimento da HP, e a influência do treinamento físico preventivo, a análise do perfil transcriptômico cardíaco nesta condição não tem sido explorado. Objetivo: nesta tese desenvolvemos 2 artigos científicos, um experimental (ARTIGO 1) e uma tradução de questionário de risco de viés (ARTIGO 2). **Objetivo** 1: analisar o efeito do treinamento preventivo no perfil de expressão gênica global do ventrículo direito na HP de ratos. Foram utilizados vinte e quatro ratos Wistar machos (206-220 g) divididos em três grupos (n=8/grupo): S, controle sedentário; SPH, hipertensão pulmonar sedentária; e TPH, grupo treinado para hipertensão pulmonar. O protocolo de treinamento preventivo foi realizado em esteira rolante por 13 semanas. A hipertrofia cardíaca por HP foi induzida experimentalmente por uma única injeção intraperitoneal (60 mg/kg) de monocrotalina após o treinamento preventivo. Os ratos foram avaliados 28 dias após a administração de monocrotalina. Peso do ventrículo esquerdo, peso do ventrículo direito e peso do átrio normalizado pelo peso corporal foram usados como índices de hipertrofia cardíaca. O perfil de expressão gênica foi realizado usando a plataforma Rat Gene ST Array. Para entender melhor a relevância biológica dos genes expressos diferencialmente, realizamos uma análise de enriquecimento funcional no contexto da ontologia gênica (processo biológico, função molecular e componente celular) e redes (KEGG, Reactome, Wikipathways e BioPlanet). **Resultados 1:** Através da análise da área de cardiomiócitos, o grupo HP apresentou hipertrofia cardíaca, e o treinamento amenizou essa alteração $p< 0,05$. O perfil de expressão gênica global identificou 687 genes diferencialmente expressos (S vs. SPH), na hipertrofia ventricular direita durante a HP, e quando comparado ao treinamento preventivo, 91 genes estavam desregulados (SPH vs. TPH). A análise da ontologia gênica nos dados de perfil de expressão revelou alteração nos genes que regulam o processo inflamatório, crescimento muscular, processos metabólicos, morte celular e comparação da regulação iônica entre SPH vs. TPH Os genes que foram alterados na fase de hipertrofia do PH e modificados pela exercício foram lúmen de grânulo alfa de plaquetas (genes Fgg e Qsox1), ligação NAD + (gene Cryl1), regulação positiva da sinalização mediada por cAMP (gene Cxcl11), regulação positiva da diferenciação de macrófagos (gene Il34), clivagem de mRNA (gene Cstf3), regulação negativa da atividade do transportador (gene Wwp2), regulação do processo do sistema respiratório (gene Mtg2) $p< 0,05$. **Conclusão 1:** O treinamento preventivo influencia mudanças no perfil de expressão gênica cardíaca na fase de hipertrofia da HP. Novos genes foram identificados, e estes podem ser biomarcadores e potenciais alvos terapêuticos na fase inicial da HP. Introdução 2: A pesquisa experimental tem aumentado e a realização de meta-análise se tornou frequente no Brasil e no mundo. Para a realização desses estudos a qualidade

metodológica é avaliada por importantes instrumentos como a ferramenta RoB-SYRCLE. **Objetivo 2**: traduzir o RoB-SYRCLE para sua utilização em estudos experimentais. **Métodos 2**: A tradução da ferramenta foi realizada seguindo um guia internacional. Após a tradução, retrotradução e aprovação da versão pelos idealizadores, a ferramenta que é composta por 10 itens, em português, foi avaliada por 15 pesquisadores com experiência em estudos animais. Os itens 1 e 2 se referem ao viés de seleção (geração de sequência, características basais e ocultação da alocação), os Itens 3 e 4 ao viés de performance; item 5 ao viés de execução (alojamento aleatório e cegamento), Item 6 e 7 ao viés de detecção (avaliação aleatória dos desfechos e cegamento), Item 8 ao viés de atrito (desfechos incompletos), item 9 ao viés de relato (relato de desfecho seletivo) e item 10 a outras fontes de viés. Os avaliadores deveriam responder em relação ao grau de compreensão (Compreendi totalmente, compreendi parcialmente, não compreendi). Análise estatística: estatística descritiva com valores percentuais. **Resultados 2:** Dos pesquisadores que avaliaram a ferramenta, 100 a 86,6% compreenderam totalmente os itens de 1 a 7, 9 e 10. Em relação ao item 8, esse foi compreendido totalmente por 80% dos pesquisadores. **Conclusão 2:** A ferramenta RoB, essa foi traduzida e utilizada com sucesso por todos os pesquisadores, mas ainda, são necessários ajustes e análise de especialistas na questão 8 devido a porcentagem de não compreensão ser de 20%.

Palavras-chave: disfunção cardíaca; hipertrofia cardíaca; hipertensão arterial pulmonary.

ABSTRACT

1 - Effect of preventive training on heart transcriptoma of rats with pulmonary hypertension
2 - Portuguese translation of the bias risk syrcle tool

Introduction 1: Induction of pulmonary hypertension (PH) by monocrotaline is common, which leads to increased right ventricular afterload, vascular remodeling, right ventricular hypertrophy and dysfunction, and ultimately heart failure. Physical exercise has long been recognized as a treatment for this condition. Although different mechanisms have been proposed to explain the development of PH, and the influence of preventive physical training, the analysis of the cardiac transcriptomic profile in this condition has not been explored. Objective: in this thesis we developed 2 scientific articles, one experimental (ARTICLE 1) and a translation of a risk of bias questionnaire (ARTICLE 2). Objective 1: to analyze the effect of preventive training on the global gene expression profile of the right ventricle in PH in rats. Twenty-four male Wistar rats (206-220 g) were divided into three groups (n=8/group): S, sedentary control; HPS, sedentary pulmonary hypertension; and TPH, group trained for pulmonary hypertension. The preventive training protocol was performed on a treadmill for 13 weeks. Cardiac hypertrophy by HP was experimentally induced by a single intraperitoneal injection (60 mg/kg) of monocrotaline after preventive training. Rats were evaluated 28 days after administration of monocrotaline. Left ventricular weight, right ventricular weight, and atrial weight normalized by body weight were used as indices of cardiac hypertrophy. Gene expression profiling was performed using the Rat Gene ST Array platform. To better understand the biological relevance of differentially expressed genes, we performed a functional enrichment analysis in the context of gene ontology (biological process, molecular function and cellular component) and networks (KEGG, Reactome, Wikipathways and BioPlanet). Results 1: Through the analysis of the cardiomyocytes area, the HP group presented cardiac hypertrophy, and the training softened this alteration $p< 0.05$. The global gene expression profile identified 687 differentially expressed genes (S vs. SPH) in right ventricular hypertrophy during PH, and when compared to preventive training, 91 genes were dysregulated (SPH vs. TPH). Analysis of the gene ontology in the expression profile data revealed changes in the genes that regulate the inflammatory process, muscle growth, metabolic processes, cell death and comparison of ionic regulation between HPS vs. TPH The genes that were altered in the PH hypertrophy phase and modified by exercise were platelet alpha granule lumen (Fgg and Qsox1 genes), NAD + binding (Cryl1 gene), cAMP-mediated signaling upregulation (Cxcl11 gene), upregulation of macrophage differentiation (IL34 gene), mRNA cleavage (Cstf3 gene), downregulation of transporter activity (Wwp2 gene), regulation of respiratory system process (Mtg2 gene) $p< 0.05$. Conclusion 1: Preventive training influences changes in cardiac gene expression profile in the hypertrophy phase of PH. New genes were identified, and these may be biomarkers and potential therapeutic targets in the initial phase of PH. Introduction 2: Experimental research has increased and meta-analysis has become frequent in Brazil and worldwide. In order to carry out these studies, the methodological quality is evaluated by important instruments such as the RoB-SYRCLE tool. Objective 2: translate RoB-SYRCLE for use in experimental studies. Methods 2: The translation of the tool was carried out following an international guide. After translation, back-translation and approval of the

version by the creators, the tool, which is composed of 10 items, in Portuguese, was evaluated by 15 researchers with experience in animal studies. Items 1 and 2 refer to selection bias (sequence generation, baseline characteristics and allocation concealment), Items 3 and 4 to performance bias; item 5 for execution bias (random placement and blinding), Item 6 and 7 for detection bias (random assessment of outcomes and blinding), Item 8 for attrition bias (incomplete outcomes), item 9 for reporting bias (report of selective outcome) and item 10 to other sources of bias. The evaluators should respond in relation to the degree of understanding (I fully understood, I partially understood, I did not understand). Statistical analysis: descriptive statistics with percentage values. Results 2: Of the researchers who evaluated the tool, 100 to 86.6% fully understood items 1 to 7, 9 and 10. Regarding item 8, this was fully understood by 80% of the researchers. The RoB tool was successfully translated and used by all researchers, but still, adjustments and expert analysis are needed in question 8 due to the percentage of non-understanding being 20%.

Keywords: cardiac dysfunction; cardiac hypertrophy; pulmonary arterial hypertension.

LISTA DE SIGLAS

μm²: micrômetro quadrado
ANOVA: Análise de variância
AT: átrios
ATP: adenosina trifosfato
ATW: Atrium weight
BW: Body weight
cDNA: DNA complementar
COBEA: Colégio brasileiro de experimentação animal
Cryl1: crystallin lambda 1
CSA: cross-sectional area
CSA: Cross-sectional área
DEG: Differentially expressed genes
DEGs: Genes diferencialmente expressos
DNA: Ácido Desoxirribonucleico
DPOC: Doença pulmonar obstrutiva crônica
FDR: false discovery rate
Foxo1: fork head box protein O1
G.O: Gene Ontology
g: Gramas
GM-CSF: multifunctional growth factor
GSK-3β: Glycogen synthase kinase-3 beta
h: horas
HE: Hematoxilina e eosina
HF: Heat failure
IAM: Infarto agudo do miocárdio
IGF-1: Fator de crescimento semelhante à insulina tipo 1
Il34: interleukin-34
kDa: quilodalton
km/h: quilômetro por hora
LL: Limiar de lactato
LV: Left ventricle
LVW: Left ventricle weight
MAPK: mitogen-activated protein kinase
Mcat: malonyl-CoA-acyl carrier protein transacylase
MCT: monocrotaline
MEF2: myocyte enhancer factor2
mg/kg: miligrama por quilo
MHz: megaherts
min: minutos
mmol/L: milimol por litro

mRNA: RNA mensageiro
ms: milissegundo
Mtg2: mitochondrial ribosome-associated GTPase 2
mTOR: mammalian target of rapamycin
NaCl 9%: solução salina
N-CoR: nuclear receptor corepressor
NF-kB: nuclear transcription factor
ºC: graus Célsius
PH: pulmonary hypertension
PI3K: fosfoinositídeo 3-quinase ou fosfatidilinositol 3-quinases
PNR: purine-rich negative regulatory
PPI: Protein–protein interaction
Qsox1: quiescin sulfhydryl oxidase 1
RM: repetição máxima
RMA: Robust Multi-array Average
RNA: Ácido ribonucleico
RT-qPCR: Reação em cadeia da polimerase em tempo real pós transcrição reversa
RV: Right ventricle
RVW: Right ventricle weight
S: Grupo Sedentário Controle
SPH: Grupo Sedentário Hipertensão Arterial Pulmonar
SRF: fator de resposta do soro
T3: Triiodotironina
TPH: Grupo Treino Hipertensão Arterial Pulmonar
TR: hormônio da tireoide
vs: versus

SUMÁRIO

ARTIGO 1

Gene expression profile of rat with compensated ventricular hypertrophy in pulmonary hypertension reveals changes following preventive training

Thaoan Bruno Mariano[1], Danielle Fernandes Vileigas[2], Paula Paccieli Freire[3], Sarah Santiloni Cury[3], Edson Assunção Mareco[4], Andreo Fernando Aguiar[5], Pedro Rocha Tenorio[6], Larissa Ferreira Rós Mariano[1], Mariana Janini Gomes, Robson Francisco Carvalho[3], Tesha Kerr[7], Ivan José Vechetti Jr[7], Francis Lopes Pacagnelli[1].

1 Postgraduate Program in Animal Science, University of Western São Paulo (Unoeste), Presidente Prudente, São Paulo, Brazil.

2 Department of Internal Medicine, Botucatu Medical School, Sao Paulo State University, UNESP, Sao Paulo, Brazil.

3 Department of Structural and Functional Biology, Institute of Biosciences, São Paulo State University, Botucatu, Brazil.

4 Postgraduate Program in Environment, University of Western São Paulo (Unoeste), Presidente Prudente, São Paulo, Brazil.

5 Center of Research in Health Science, North University of Paraná (Unopar), Avenue Paris, 675, Jardim Piza, Londrina, PR 86041-120, Brazil

6 Physiological Science Program, State University of Londrina, Londrina, PR, Brazil

7 Department of Nutrition and Health Sciences, University of Nebraska-Lincoln, Lincoln, NE, USA

Abstract

Background: Clinical studies have shown that physical exercise is protective against cardiac changes with established pulmonary hypertension (PH). In contrast, there is substantially less information available on whether prior exercise offers any protection in situations where, despite regular exercise, a serious condition characterized as PH occurs. Most experimental work has focused on the effects and molecular mechanisms underlying physical exercise in

the heart failure phase, rather than the compensated hypertrophy phase in PH. This study aimed to analyze the effect of preventive training on the global gene expression profile of the PH-compensated hypertrophy phase of twenty-four male Wistar rats, divided into three groups (n=8/group): S, sedentary control; SPH, sedentary pulmonary hypertension; and TPH, Training Pulmonary Hypertension group. All rats underwent a two-week adaptation period; TPH group rats then proceeded to an eight-week training period on a treadmill. At the beginning of the 11th week, S group received an intraperitoneal injection of saline, and SPH and TPH groups received an injection of monocrotaline (60 mg/kg). Rats in the TPH group then continued with the training protocol until the 13th week. Cardiac hypertrophy by PH was experimentally induced by a single intraperitoneal injection (60 mg/kg) of monocrotaline after preventive training. Gene expression profiling was performed to better understand the biological relevance of differentially expressed genes. Results: Through the analysis of the cardiomyocyte area, the PH groups had cardiac hypertrophy, and training mitigated this change $p< 0.05$. Global gene expression profiling identified 687 differentially expressed genes (S vs. SPH), during hypertrophy caused by PH in the right ventricle, and when compared to preventive training, 88 genes were dysregulated (SPH vs PHT). Gene enrichment analysis between SPH and PHT, $p<0.05$, on expression profiling data revealed alteration on genes that regulate metabolic processes (*Cryl1, Mtg2*) and cell death and proteolysis (*Il34, Qsox1*). Conclusion: Preventive training influences changes in cardiac gene expression profile in the hypertrophy stage of PH. Our results have identified potential biomarkers and therapeutic targets in the early stage of PH.

Keywords: Cardiac dysfunction. Cardiac hypertrophy. Pulmonary arterial hypertension. Preventive training.

Background

Changes such as persistent vasoconstriction, structural remodeling of the pulmonary arteries, reduced microvascular cross-sectional area, increased vascular resistance and endothelial dysfunction are characteristic of pulmonary hypertension (PH) [1]. Right ventricular (RV) hypertrophy is caused by a pressure overload attributed to PH, which can lead to RV dysfunction and Right Heart Failure, increasing morbidity and mortality [2-4]. In patients with PH who have changes in the pulmonary arteries, survival is related to RV function [5].

In a mouse model of monocrotaline-induced PH, adaptations caused by pressure overload change gene expression, which is associated with the severity of PH [6]. These

adaptations demonstrate that compensated RV hypertrophy leads to unique gene profile, changing specific pathways such as MAPK signaling and apoptosis, which are important for RV hypertrophy [7,8].

In patients with PH, physical exercise has been indicated for promoting benefits in cardiac remodeling, described in human and animal models of RV hypertrophy, by altering the proteins involved in some pathways, such as mTOR, PI3K, IGF-1 and GSK-3β. [9,11]. In addition, exercise has been highlighted as a therapeutic treatment for cardiac protection in PH by improving cardiac function, reducing cardiac hypertrophy and proteins such as the NF-kB, and attenuating PH development without interfering in mRNA-encoding myosin and collagen expression during PH [12-14].

Despite these well-established adaptations, a global profile of genes related to cardiac protection of exercise in PH in the phase of compensated hypertrophy is still missing. Therefore, we aimed to investigate the influence of preventive aerobic training on the gene expression profile in rats with RV hypertrophy by PH and to define new molecular interaction networks of heart tissue that regulate training-mediated cardiac protection.

Results

Training decreases cardiac hypertrophy

After 28 days of MCT injection, SPH and TPH group exhibited cardiac hypertrophy in right atrium (S: 0.21g ± 0.031g; SPH: 0.36g ± 0.17g; TPH: 0.35 ± 0.11; $p= 0.0031$) and right ventricle (S: 0.43g ± 0.06g, SPH: 0.82g ± 0.30g; TPH: 0.46 ± 0.06; $p= 0.0042$). MCT-treated rats develop a significant increase in cardiomyocyte cross-sectional area (CSA; taken as an index of muscle hypertrophy). The preventive exercise promoted a significant reduction in hypertrophy in TPH animals (S: 62.43 ± 6.56 μm^2; SPH: 106.8 ± 22.59 μm^2; TPH: 87.65 ± 4.52 μm^2; $p< 0.001$). (Fig. 1).

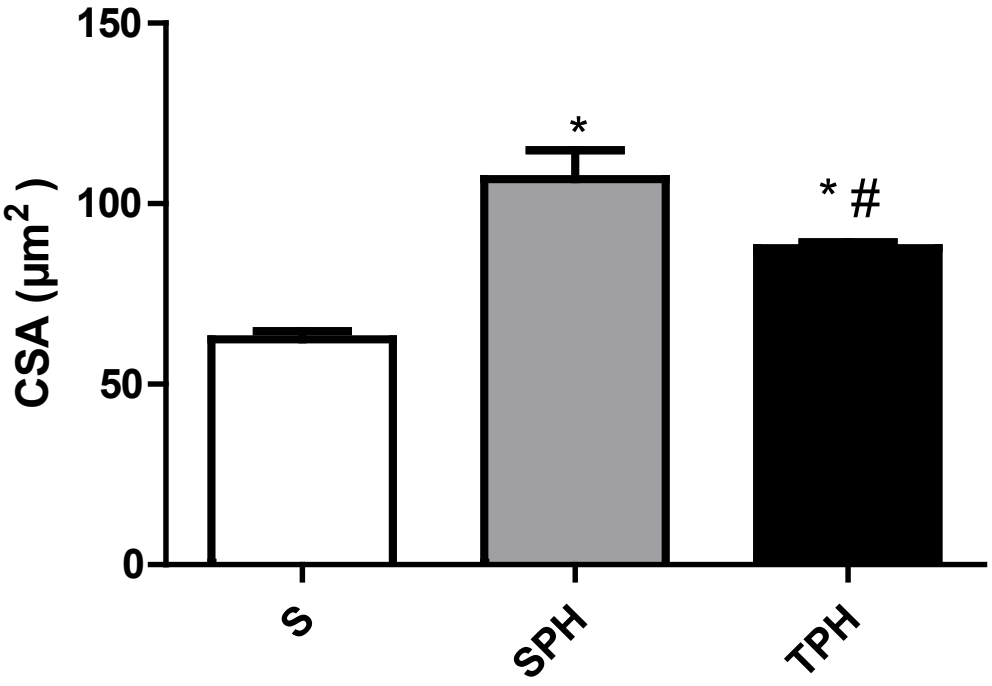

Figure 1. Monocrotaline-treated rats develop right ventricle hypertrophy. Preventive exercise decreases cross-sectional area (CSA; μm^2). S (n = 8): Sedentary Control; SPH (n = 8): Sedentary Pulmonary Hypertension; TPH (8): Pulmonary Hypertension with Training; different letters, significant difference $p<0.05$. ANOVA and Tukey's post hoc.

Venn diagram highlights the overlapping genes in disease and with training

Venn's diagram was used to identify variation in gene expression between the sedentary, sedentary pulmonary hypertension and training pulmonary hypertension groups. In an analysis of up-regulated genes in PH, we observed 26 genes exclusive expressed in training group (Figure 2A). On the order hand, the same analysis in the down-regulated genes in PH showed 59 genes exclusive altered in training group (Figure 2B). This analysis does not represent the absence of expression on this group of genes. However, it may represent a differential expression pattern of these genes. Venn's diagram also demonstrated that TPH group had major amount of differently expressed genes among the groups.

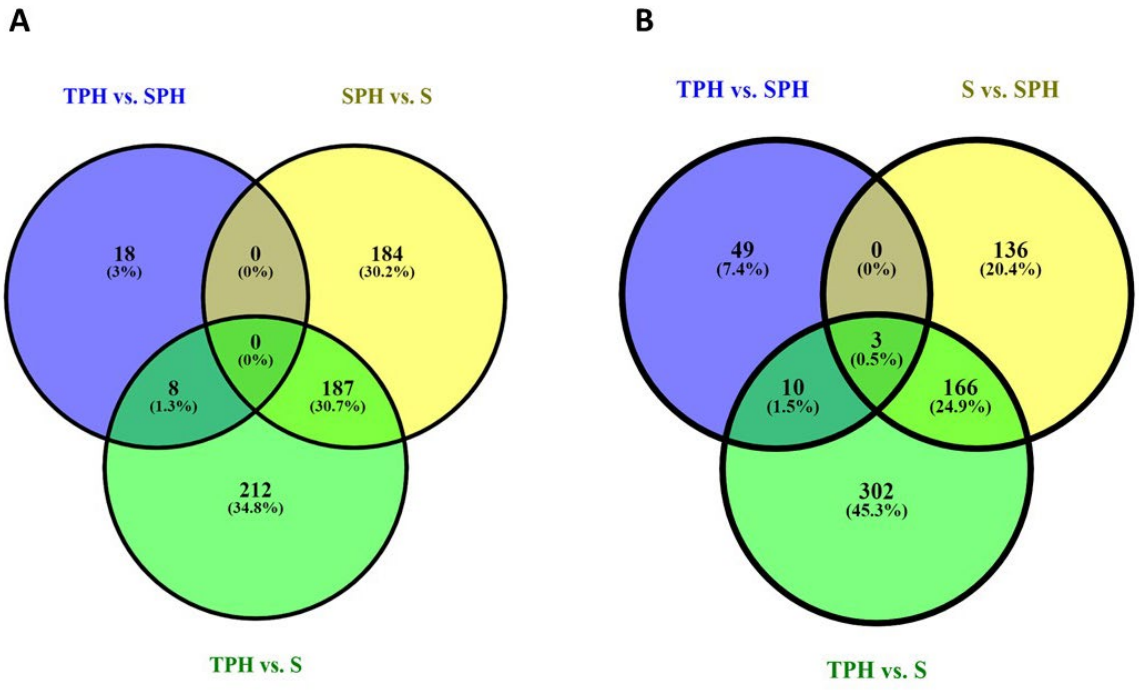

Figure 2. Venn diagrams of genes with significantly different expression in S, SPH and TPH groups microarray comparisons. A. The Venn diagrams show the number of overlapping and nonoverlapping up-regulated genes in each of the three groups. B. The Venn diagrams show the number of overlapping and nonoverlapping down-regulated genes in each of the three groups.

Hierarchical clustering of the 88 genes that stand out in TPH

To identify the hierarchical clustering of differentially expressed genes up and down we performed a heat map analysis. The expression profile of differentially expressed genes for preventive training is shown in Figure 4.

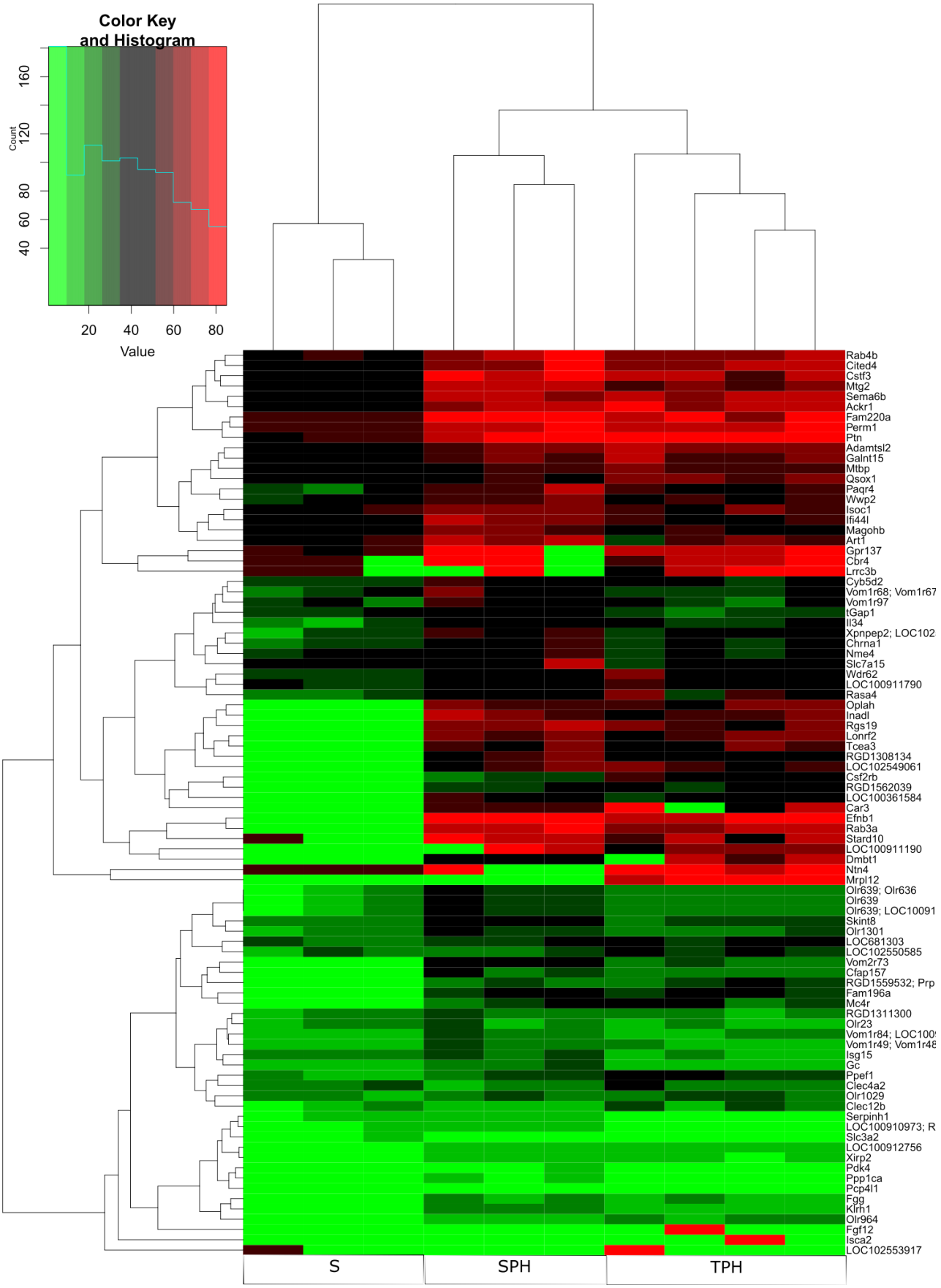
Color Key
and Histogram
Count
160
120
80
60
40
20
40
60
80
Value
Rab4b
Cited4
Cstf3
Mtg2
Sema6b
Ackr1
Fam220a
Perm1
Ptn
Adamtsl2
Galnt15
Mtbp
Qsox1
Paqr4
Wwp2
Isoc1
Ifi44l
Magohb
Art1
Gpr137
Cbr4
Lrrc3b
Cyb5d2
Vom1r68; Vom1r67
Vom1r97
tGap1
Il34
Xpnpep2; LOC102
Chrna1
Nme4
Slc7a15
Wdr62
LOC100911790
Rasa4
Oplah
Inadl
Rgs19
Lonrf2
Tcea3
RGD1308134
LOC102549061
Csf2rb
RGD1562039
LOC100361584
Car3
Efnb1
Rab3a
Stard10
LOC100911190
Dmbt1
Ntn4
Mrpl12
Olr639; Olr636
Olr639
Olr639; LOC10091
Skint8
Olr1301
LOC681303
LOC102550585
Vom2r73
Cfap157
RGD1559532; Prp
Fam196a
Mc4r
RGD1311300
Olr23
Vom1r84; LOC100
Vom1r49; Vom1r48
Isg15
Gc
Ppef1
Clec4a2
Olr1029
Clec12b
Serpinh1
LOC100910973; R
Slc3a2
LOC100912756
Xirp2
Pdk4
Ppp1ca
Pcp4l1
Fgg
Klrh1
Olr964
Fgf12
Isca2
LOC102553917
S
SPH
TPH

Figure 3. Groups S, SPH, and TPH hierarchical clustering analyses (Heatmap) using unsupervised Euclidean distance of all differentially expressed genes between the groups showed by Z-scores Genes are represented in rows and groups in columns.

Interaction networks and gene ontology enrichment analysis.

In order to understand the interaction networks and ontology changes associated with the preventive training in PH, we performed a global mRNA expression profiling assessment. The analysis identified 687 differentially expressed genes in the SPH compared to S ($p \leq 0.05$ and fold change ≥ 1.5 and ≤ -1.5), of which 308 and 379 were up- or down- regulated, respectively. Comparing preventive exercise to sedentary lifestyle, (SPH vs TPH), 88 genes were differentially expressed ($p \leq 0.05$ and fold change ≥ 1.5 and ≤ -1.5), of which 26 and 62 were up- or down- regulated, respectively.

The selection criteria for validation by RT-PCR were the genes with the greatest fold change alteration which were present in the comparisons between all groups and according to the important metabolic function in PH. These genes were *Cryl1* (-5.14 fold chang), *Il34* (-2.05 fold change), *Qsox1* (2.89 fold change) and *Mtg2* (-2.12 fold change).

To determine the biological and functional implications of gene expression changes in PH and preventive training, we performed functional enrichment of the differentially expressed genes. The functional class was assigned to the 88 (SPH vs TPH) (Fig. 5).

This analysis showed that the ontologies were grouped into critical biological processes for PH and training. And the genes validated by RT-PCR are related to the networks of positive regulation of macrophage proliferation (*Il34*), regulation of respiratory system process (*Mtg2*), platelet alpha granule lumen (*Qsox1*) and glucuronate catabolic process (*Cryl1*) (Fig. 5). There were 23 interaction networks were clustered in biological processes important for right heart hypertrophy including pentose and glucuronate interconversions (*Cryl1*), Interleukin-5 regulation of apoptosis (*Qsox1*), signaling by Interleukins (*Il34*) and U251 Mitochondria M1 (*Mtg2*) (Fig. 6). The genes are related to metabolic processes (*Cryl1, Mtg2*) and cell death and proteolysis (*Il34, Qsox1*).

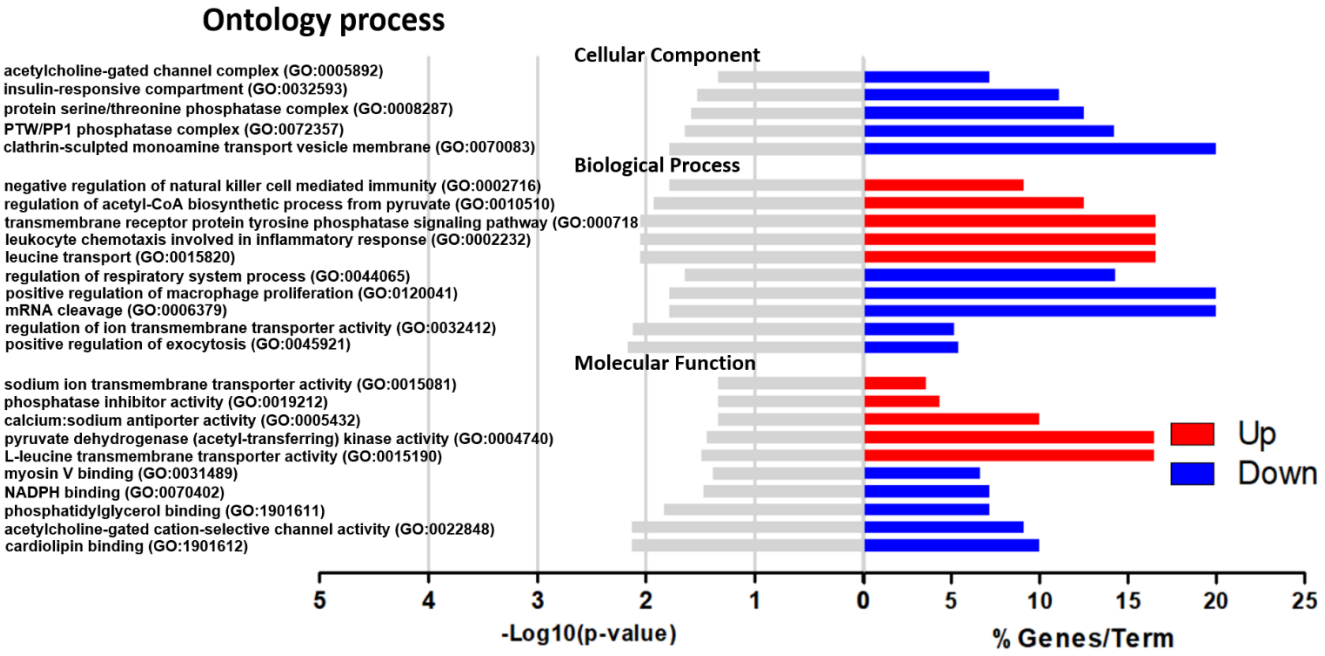

Figure 5. Gene ontology (G.O.) enrichment analysis of up and down differentially expressed genes in cardiac tissue of TPH group compared to SPH group. The analysis was performed using the Enrichr, providing the significantly enriched GO terms Molecular Function, Biological Process, and Cellular Component. On the left panel, the names of the gene ontology process. On the right panel, changes are displayed as the percent of increased and decreased genes presented in the data set compared to the total number of genes in each pathway (horizontal axis). Top interaction networks identified with up-regulated genes and down-regulated genes (p adjust-value < 0.05).

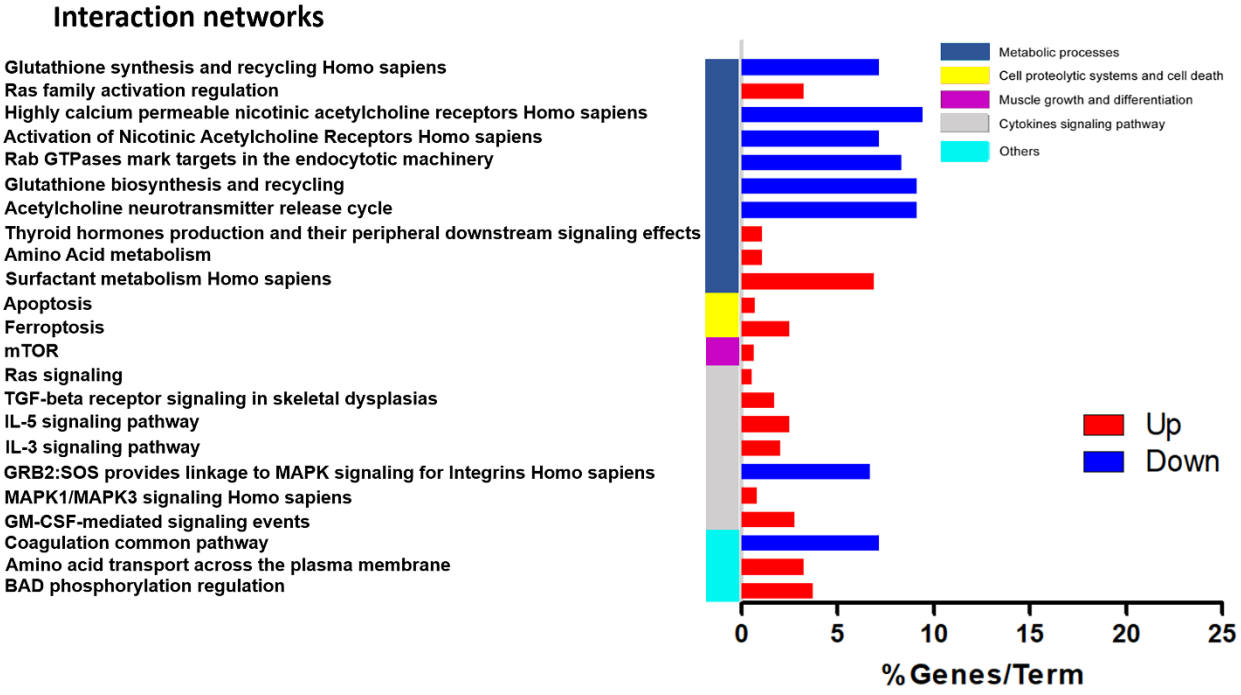

Figure 6. TPH up and down regulated process compared with SPH. On the left panel, the interaction networks. On the right panel, changes are displayed as the percent of increased and decreased genes presented in the data set compared to the total number of genes in each pathway (horizontal axis). Top interaction networks identified with upregulated genes and down-regulated genes (p adjust-value < 0.05).

Protein-Protein Interaction Network in Muscle cardiac in right ventricle dysfunction with preventive training.

Complex interaction network analysis of deregulated genes between SPH and TPH in right ventricular hypertrophy is illustrated in Figure 7.

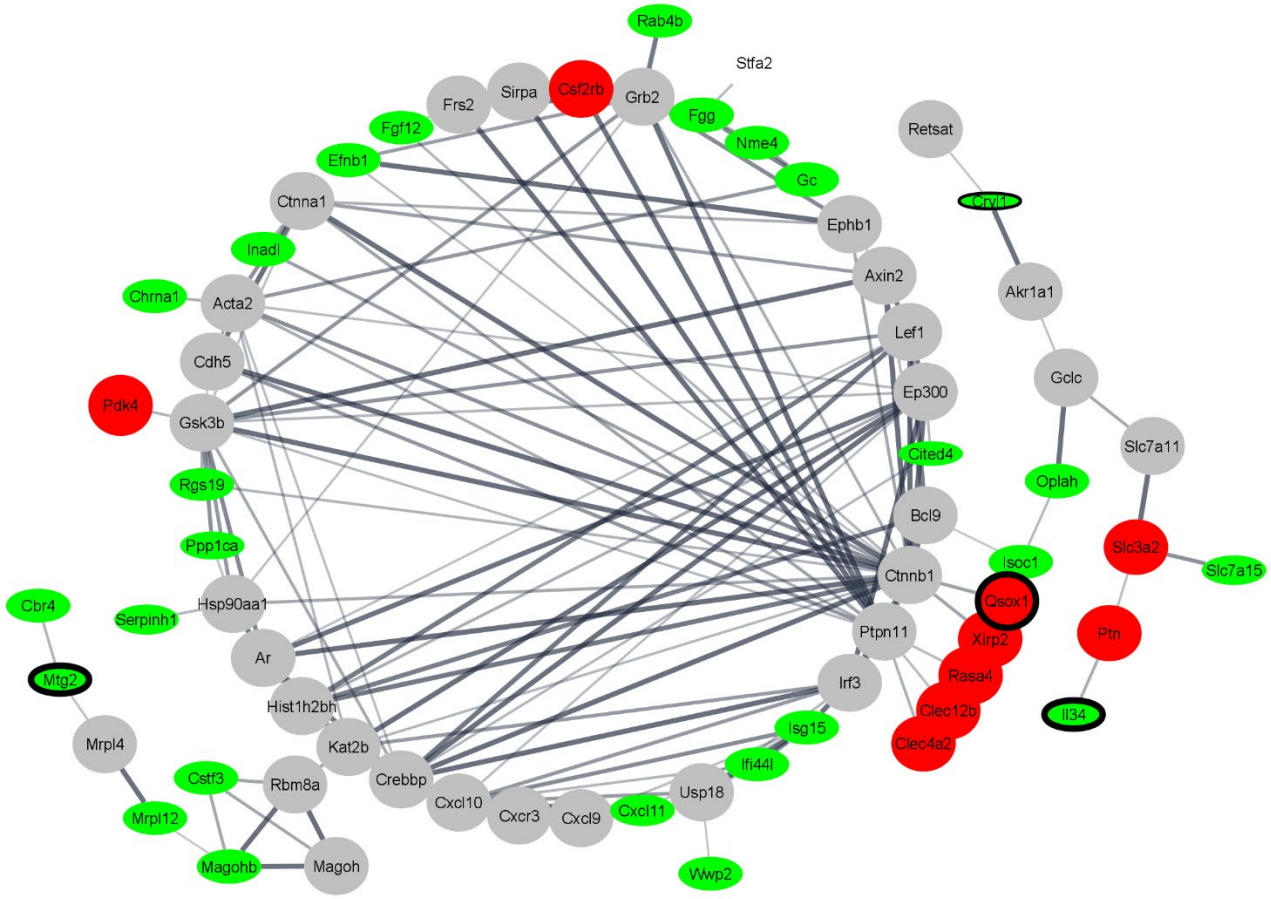

Figure 7. Aprotein–protein interaction (PPI) network comparing TPH and SPH groups in cardiac muscle in right ventricular dysfunction. A black circle around a gene indicates validation by PCR. The thickness of the grid lines show a the amount of co-expression. The red circles show the largest, and the green circles the smallest fold change according to their size.

Cardiac hypertrophy in pulmonary hypertension is associated with changes in *Cryl1, Il34, Qsox1* and *Mtg2* gene expression

Our data highlight, for the first time, a set of genes that targets transcripts that encode inflammatory (*Il34*), mitochondrial (*Mtg2* and *Cryl 1*) and homeostasis (*Qsox1*) proteins in cardiac hypertrophy during pulmonary hypertension.

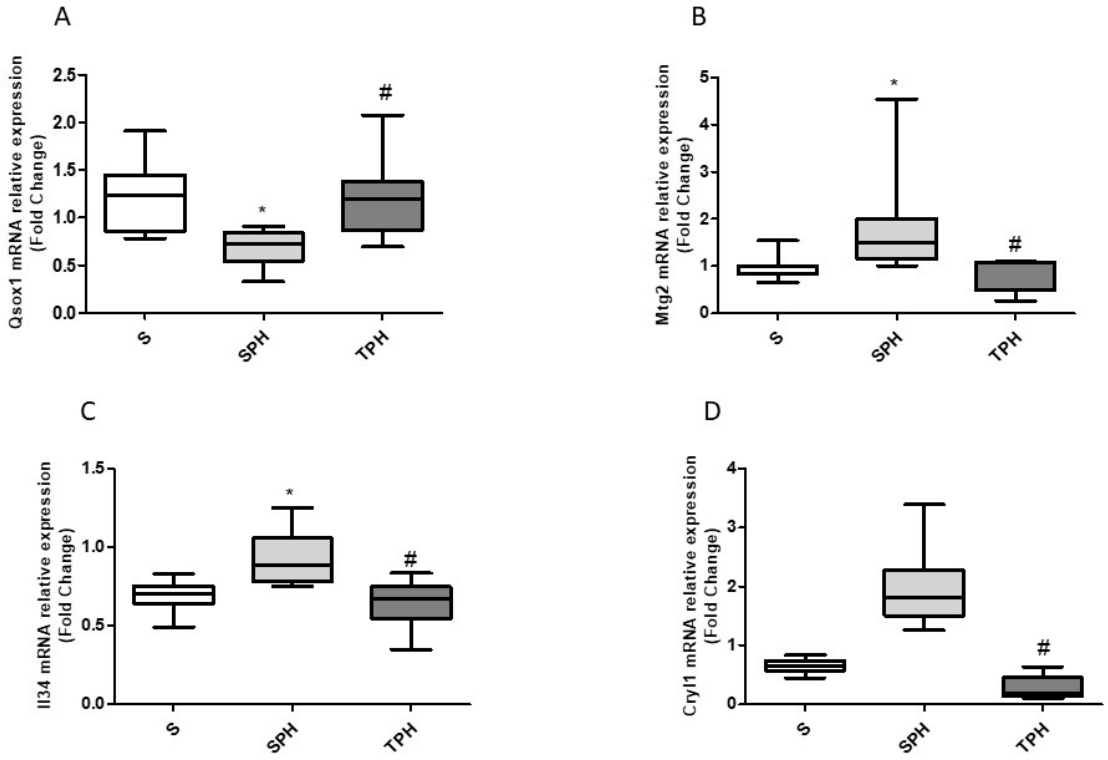

Figure 8. RT-PCR analysis of *Qsox1* (A); *Mtg2* (B); *Il34* (C) *and Cryl1* (D). S: Sedentary Control; SPH: Sedentary Pulmonary Hypertension; TPH: Pulmonary Hypertension with Training; * p<0.05 compared
to S; # compared to SPH. Kruskal-Wallis test and Dunn's post hoc.

Discussion

Our results highlight changes in interaction networks and ontology in crucial regulatory processes involved in heart hypertrophy in PH and the vital function of preventive exercise. Specifically, our results suggest that key of DEGs may contribute to cardiac hypertrophy in PH and preventive exercise attenuated this alteration.

Our group has previously demonstrated that preventive training increases pulmonary artery acceleration time/pulmonary ejection time ratio and decreases Fulton's index and cross-sectional areas of myocyte cells [12,14]. This indicates that preventive exercise reduces RV pressure overload.

Cardiac hypertrophy is an adaptation of the ventricle to overload that can lead to heart failure [22]. Recent studies indicate that cardiac hypertrophy causes changes to the

metabolic and contractile functions of the heart and also found that fetal genes were expressed in this condition. These studies corroborate our findings as they show that changes in gene expression occur in this phase of cardiac hypertrophy [23,24]. Gene ontology and enrichment analysis revealed several genes that regulate metabolic processes, cell death, and proteolysis that were changed after induction of PH. *Cryl1, Mtg2, and Il34* were increased in the disease state , and were decreased in sick animals that trained. The *Qsox1* gene was reduced in the disease state and increased with training.

The MCT treatment induces PH, as demonstrated in a previous study by our research group [12]. These rats increased in cardiomyocyte cross-sectional area, had atrial and right ventricular hypertrophy, as well as severe pulmonary hypertension [12]. Studies with a monocrotaline-induced HP model also identified right ventricular hypertrophy [9,12,14], which corroborates our findings. We showing improvement in RV hypertrophy cardiac exercise training was found to be beneficial in stable PH but detrimental in progressive PH. Although Handoko et al. 2009 [25] did not corroborate our findings, other studies do, showing similar improvement in cardiac hypertrophy with training [13,26].

Among some altered genes the *Cryl1 (*Crystalin Lambda 1) was one of them. This gene encodes the mitochondrial CRY protein which is related to the Xylulose-5-phosphate formation and metabolism pathways [27,28]. Most studies have evaluated this gene in cases of liver cancer [28] or obesity but not to training [29,30]. To our knowledge, the *Cryl1* gene in pulmonary hypertension has not yet been evaluated. Mitochondrial damage is believed to be a crucial element of pulmonary hypertension, changes in mitochondria generate metabolic changes in the aerobic glucose pathway due to the low level of O2, there are also changes in the reactive oxygen enzymes ROS, nitric oxide NO/HIF-1 and mitochondrial Ca+2 transit (31). In our findings, this gene is increased in the disease, and preventive training was able to reduce its expression. Another gene is *Mtg2* (Mitochondrial Ribosome Associated GTPase 2), which gives rise to one of the three GTPase proteins that are part of the G protein superfamily, whose function is the biogenesis of the mitochondrial ribosome [32]. In a study that used mitochondrial sequencing, they mention that the *Mtg2* gene can negatively regulate mitochondrial translation, and this implies the response to physical training [33]. Therefore, it influences cellular respiration. Data from our study point to an increase in the *Mtg2* gene in the hypertrophy phase of PH, which may indicate a compensation mechanism, and preventive training was able to mitigate this change. So far, this gene has not been described in cardiac muscle alterations in PH, nor in preventive training, which suggests that it is a biological marker of this condition.

Studies with a global analysis of genes in HF due to aortic obstruction and in PH identified changes in genes associated with cardiac macrophages (*Il34*) and fibroblasts due to pressure overload [34,35]. The protein encoded by the *Il34* gene is a cytokine that controls the production, differentiation, and function of macrophages. Interleukin-34 protein (IL-34) is one of the interleukins discovered in recent years that is expressed in patients with coronary heart disease, which induces inflammatory processes and atherosclerosis [36]. Mathew et al. 2020 also did a global gene analysis in animals with PH, but looked at genes from the lungs and not the heart. They found that animals with PH express genes related to inflammation, cell proliferation, vascular smooth muscle contraction, and they also highlighted mitochondrial damage and activation of antioxidants [37].

We find increased in *Il34 gene expression* in the cardiac hypertrophy phase in PH. IL-34 is a new member of the cytokine family, which is physiologically expressed in different tissues of the body, in inflammatory processes, autoimmune diseases, and cancer [30]. In recent studies, Interleukin 34 (*Il34)* has been identified as a new biomarker for heart failure, in chronic situations or in acute ischemia, and positively regulates the defense cell interaction networks of monocytes and macrophages [38]. Our study indicates that this interleukin is released by the heart in the cardiac hypertrophy phase triggered by PH, and in previously trained animals the expression is reduced. It can be considered a biomarker of the cardioprotective effects of exercise.

Quiescine sulfhydryl oxidase 1 (*Qsox1*) is an enzyme of the multidomain thioredoxin superfamily secreted by fibroblasts [39]. Among other potential functions, *Qsox1* supports extracellular matrix assembly in fibroblast cultures, suggesting that matrix metalloproteinases proteins may be a substrate for *Qsox1* [39]. In another study, the increase in this gene promoted cardio protection, in which it helped to maintain cardiac function, through an endo/sarcoplasmic reticulum (ER/SR) protein, which in the acute response to iso stress maintained adequate ventricular contraction as a protective response. This indicates that this gene plays an important role in cardiomyocyte homeostasis and function, as well as assisting in the adaptive response of the heart to acute stress [40]. Until the present study, there were no reports about this gene in relation to training and pulmonary hypertension. We found that *Qsox1* decreased in PH, but increased in the trained group, and this may have promoted cardio protection by improving cardiac function.

Structural abnormality characteristics and global gene expression profiling analysis have been described previously in PH [41]; in this study the authors identified new potential candidate genes in cardiac hypertrophy in PH: *Ldha, Slc2a1, Prkaa, Igf2, Bnip3l, Vegfa, Flt1* and *Gata2,* implicated numerous biochemical and molecular interaction networks in disease

onset and progression, developed gene signatures to appropriately classify types of pulmonary hypertension and severity of illness, and identified novel gene mutations leading to hereditary forms of the disease. In our results, we found genes in the PH phase, in addition to discovering the effect of preventive training on these genes.

Elevated pulmonary arterial pressure and increased pulmonary vascular resistance put excess stress on the right ventricle (RV), resulting in much of the morbidity and mortality attributed to pulmonary hypertension. Therefore, abundant gene expression profiling has focused on changes in the myocardium and adaptation of the right heart to stress through pressure overload and hypoxia-induced pulmonary hypertension. A study with mice has revealed that gene expression profile changes after exposure to moderate chronic hypoxia predominated in the right ventricle compared to the left ventricle (LV) [42]. These changes in the RV were associated with metabolic networks (*Pdk4, Car3, Qsox1, Rasa4, Chrna1, Rab4b, Rab3a*, and *Mtg2*), cell proteolytic system and cell death (*Clec12b, Qsox1, Csf2rb*), and transcription (*Ppp1ca*). A majority of the upregulated genes were also identified as targets of HIF1α, the major transcription factor related to hypoxia known to have important implications in the development and progression of PH [43].

In summary, we showed in compensated hypertrophy phase genes that may provide markers for the early prediction of clinical outcome in PH as well as potential targets for early intervention

Study limitations

The isolated assessment of mRNA expression is not related to protein expression (they are intrinsically linked). Some data were previously published by the group of researchers

Conclusion

The present study indicates that preventive training protects the myocardium by reducing cardiac hypertrophy, and regulates alterations in genes such as: *Qsox1, Il34, Cryl1* and *Mtg2* which is related to inflammation, mitochondrial activity and homeostasis. Our results have identified potential biomarkers and therapeutic targets in the early stage of PH

Methods

Animals and experimental design

All experimental protocols used in this study were conducted in accordance with the principles of laboratory animal care formulated by the Brazilian College of Animal Experimentation (COBEA) and according to the Guide for the Care and Use of Laboratory Animals published by the National Research Council [17]. All procedures were approved by the Ethics Committee of the University of Western Sao Paulo–UNOESTE (numbers: CEUA 2484).

To conduct this study, 24 male Wistar rats were used at two months of age and an average weight of 206–220 g, from the Central Animal Facility of the University of Western São Paulo, São Paulo, Brazil. The animals were kept in the Animal Experimentation Laboratory of the same institution, in plastic cages with dimensions of 41x34x16 cm (two animals/cage) at a temperature from 21 to 23°C and relative humidity of 50–60%, with luminosity cycles of 12 h (light/ dark) starting with the light cycle at seven am. The rats received food in a controlled manner (Alisul; Supralab, São Leopoldo, RS, Brazil) and water *ad libitum*.

The rats were randomly using random colors in your tails distributed into three experimental groups of eight animals each, denominated as sedentary control (S), sedentary pulmonary hypertension (SPH), and trained pulmonary hypertension (TPH).

Preventive Training

The animals of the TPH group were submitted to an aerobic training protocol performed on a treadmill adapted for rodents (model TK 1, IMBRAMED). The protocol consisted of 13 weeks with a frequency of training five times a week, 10 weeks before the application of monocrotaline (2 weeks of adaptation and 8 weeks of training) and 3 weeks after injection of monocrotaline [12, 14]. In adaptation, there was a gradual increase in speed and time, starting at a speed of 0.6 km/h for 15 min on the first day and ending at a speed 0.9 km/h for 45 min at the end of the second week [12]. A 5-minute warm-up and cool-down period was included in each session at a speed of 0.6 km/h. At the beginning of the 11th week of training, 24 h after the TPH group animals received the injections, the lactate threshold was performed to determine the intensity. This speed was adjusted after performing more tests to assess the lactate threshold in the 12th and 13th week. The exercise intensity at the 11th week was 60 min at 0.8 km/h. The last week it was 0.9 km/h for 60 min [12, 14].

Induction of pulmonary hypertension

In the 10th week, the protocol for induction of PH was performed in the rats of the SPH and TPH groups with an intraperitoneal injection of a single 60 mg/kg monocrotaline

dose (PHL89251; Sigma Chemical, St Louis, United States), a single saline dose (NaCl 0.9%) was administered intraperitoneally to the S group to ensure that all rats were subjected to the same degree of stress. [14, 18].

Incremental training test

24 hours after the application of monocrotaline, rats in the TPH group were submitted to incremental stress tests in the 11th, 12th, and 13th weeks to adjust the exercise speed [13, 14].. The tests started with a warm-up at 0.5 km/h, followed by five minutes of rest, the speed followed increases of 0.2 km/h every three minutes until the lactate reached a 1 mmol/l comparative value or exhaustion. Exhaustion was established when the animals were unable to run for up to three minutes. After each increase in speed, the rats were manually removed from the exercise area for one minute for blood collection. Blood samples were taken from rat tails every three minutes. An Accutrend Plus lactometer (Roche, Barcelona, Spain) was used. The device was calibrated according to the manufacturer's specifications. An arithmetic mean of all velocities in the experimental group up to the lactate threshold or exhaustion was performed to determine the training velocity[16]. We considered the lactate threshold to be when there was no lactate increase of 1.0 mmol/l above the blood lactate concentration [17,12].

Two weeks after completing the exercise protocol, the rats were assessed for tachypnea, and weighted, anesthetized with intraperitoneal sodium pentobarbital (50 mg/Kg) injection, and euthanized by decapitation. The signs of heart failure were assessed: pleural and pericardial effusions and the presence of lung and liver congestion. Fragments of liver and lung were weighed before and after drying sessions (65 °C for 72 h) to evaluate wet/dry weight ratios. Left ventricle weight (LVW), right ventricle weight (RVW), and atrium weight (ATW) normalized by body weight (BW) were used as indexes of heart hypertrophy. The heart was excised, immediately frozen in liquid nitrogen, and stored. The RV was divided in two, half frozen immediately in liquid nitrogen at −80 °C and the other part fixed in 10% buffered formalin.

Morphometric procedures

The right ventricle was fixed in 10% buffered formalin solution for 48 hours. After fixation, the tissues were placed on paraffin blocks. The histological sections (4 μm thick) from S (n = 8), SPH (n = 8) and TPH (n = 8) were obtained in an automatic microtome RM2265 (Leica Microsystems, Wetzlar, Germany) to determine cross-sectional area (CSA), using haematoxylin–eosin solution (HE). At least 50 cardiomyocyte CSA were measured from each RV using a LEICA microscope (model DM750, Leica Microsystems, Wetzlar,

Germany). After choosing sites with the most cells on a cross-section, different fields were captured and analyzed. The selected myocytes were cross-sectioned, had a round shape and visible central nucleus, and were located in the subendocardial layer of the RV muscular wall. All images were captured by video camera at 40X objective with 400x magnification [12,14].

RNA preparation

Total RNA was extracted using TRIzol reagent (Life Technologies, USA) as described by the manufacturer. Total RNA was solubilized in nuclease free-water and treated to eliminate genomic DNA contamination with DNA-free kit (Life Technologies, USA) as described by the manufacturer. Total RNA quantity was determined by the A 260 nm/A 280 nm and A 260 nm/A 230 nm ratios (acceptable when both ratios were > 1.8). RNA Integrity was ensured by obtaining a RNA Integrity Number - RIN > 8 with Agilent 2100 Bioanalyzer (Agilent Technologies, Germany).

Global gene expression profiling analysis

Gene expression profiling was performed using the Rat Gene 1.0 ST Array platform (Affymetrix, USA) in Experimental Research Unit (UNIPEX) Unesp Botucatu, that covers 17,061 RefSeq transcripts, according to the manufacturer's instructions. The Ambion W.T. Expression Kit (Life Technologies, Carlsbad, CA, USA) was used for cDNA synthesis and cRNA amplification, while the fragmentation and labeling procedures were performed with the Affymetrix GeneChip W.T. Terminal Labeling Kit. Array's hybridization, washing and scanning, were carried on the Affymetrix GeneChip Hybridization Oven 645, Fluidic Station 450 and Scanner 3000 7 G, respectively. Quality control and probe set summarization to attain gene-level signal data was provided by Affymetrix Expression Console software. Data analysis was performed with the R language (v.2.13.0). Background correction and quartile data normalization were applied using RMA (Robust Multi-array Average) algorithm 70. The limma Bioconductor package71 was used to identify differentially expressed genes (DEG). Cutoffs for significant changes were a fold-change > 1.5 and a p-value ≤ 0.05. A list with all genes and comparisons between groups can be found in supplementary material 1.

Interaction networks and gene ontology enrichment analysis

To further understand the biological relevance of differential expressed genes, we performed functional enrichment analysis in the context of the Interaction networks and Gene Ontology (G.O.) categories, KEGG, Reactome, WikiPathways, BioPlanet, Biological Process, Molecular Function and Cellular Component (https://maayanlab.cloud/Enrichr/). A p-value cut-off of 0.001 was used to identify enriched processes. A kappa score was calculated to

reflect the relationships between the terms based on the similarity of their associated genes. Lick web services, with the threshold set at 0.3. was used to provide a comprehensive view on the relevant interaction networks using experimental and *in silico* data from gene networks, protein–protein interactions, and functional interactions networks were visualized and analyzed with the online STRING database (https://string-db.org) version 11.0. All STRING network analyses were performed with a medium confidence level (0.4). To perform the clustering between all groups, the Morpheus website was used, https://software.broadinstitute.org/morpheus, an excel table was prepared with only the genes and their respective values.

Real-time polymerase chain reaction after reverse transcription (RT-PCR)

The High-Capacity Reverse Transcriptional Kit (ThermoScientific) was used for the synthesis of complementary RNA (cDNA) from 1000 ng of total RNA for each sample. Using real-time quantitative PCR (qPCR), cDNA was used to evaluate the relative levels of Rattus norvegicus crystallin lambda 1 (*Cryl1*) mRNA, Rattus norvegicus interleukin-34 (*Il34*) mRNA, Rattus norvegicus mitochondrial ribosome-associated GTPase 2 (*Mtg2*) mRNA, Rattus norvegicus quiescin sulfhydryl oxidase 1 (*Qsox1*) mRNA. The Taqman™ Universal Master Mix II (AppliedBiosystems, Foster City, U.S.) and the StepOne Plus system (ThermoScientific) were used for qPCR. All samples were analyzed in duplicates. The cycling conditions were at 50 °C for two minutes and 95 °C for ten minutes. This was followed by 40 cycles of denaturation at 95 °C for 15 seconds and the final extension at 60 °C for one minute. Gene expression was quantified relative to the values of the S group after normalization by expression levels of the beta-actin reference gene (Actb) using the 2 ^ -DDCt method (Livak et al., 2001). Taqman assay number of genes *Cryl1* (Rn00598394_m1), *Il34* (Rn01432377_m1), *Mtg2* (Rn01416052_m1), *Qsox1* (Rn00584808_m1), and *Rasa4* (Rn01481905_m1) were selected from (thermofisher.com). PCR validation results in supplementary material 2.

Statistical analysis

Statistical analyses were performed using GraphPad Prism software (Graph-Pad Software, La Jolla, U.S.). The Shapiro-Wilk test was used to assess data normality. To analyze the genes data, ANOVA and Tukey's post hoc were used with data parametric. Kruskal-Wallis test and Dunn's post hoc were used with data non-parametric. The significance level was considered when $p < 0.05$.

Three animals were used for the S group (animals chip number 1, 2, 3), three animals for the SPH group (animals chip number 6, 19, 37) and four animals for the TPH group (animals chip number 45, 50, 57, 61). For the selection of these animals, the RIN criterion was used, being above 6. All chips were normalized using the Robust Multi-array Average (RMA) method implemented in Array Studio. All data processing was performed using Array Studio software. Mean expression levels were obtained by calculating the geometrical means of the RMA-normalized data for pulmonary hypertension and control groups. A two-sided t-test was performed using the inference module of Array Studio, to determine which genes were significantly differentially expressed between the pulmonary hypertension and control groups, and Benjamini–Hochberg false discovery rate (FDR) multiple testing correction and alpha level of 0.05 was applied.

Funding

This study was supported by São Paulo Research Foundation (FAPESP, grant #2016/11344-0, FLP).

References

1 Poch D, Mandel J. Pulmonary Hypertension. Ann Intern Med. 2021;174(4): ITC49-ITC64. doi: 10.7326/AITC202104200.

2 Elia D, Caminati A, Zompatori M, Cassandro R, Lonati C, Luisi F, Pelosi G, Provencher S, Harari S. Pulmonary hypertension and chronic lung disease: where are we headed? Eur Respir Rev. 2019 21;28(153):190065. doi: 10.1183/16000617.0065-2019.

3 Konstam MA, Kiernan MS, Bernstein D, Bozkurt B, Jacob M, Kapur NK, Kociol RD, Lewis EF, Mehra MR, Pagani FD, Raval AN, Ward C; American Heart Association Council on Clinical Cardiology; Council on Cardiovascular Disease in the Young; and Council on Cardiovascular Surgery and Anesthesia. Evaluation and Management of Right-Sided Heart Failure: A Scientific Statement From the American Heart Association. Circulation. 2018 15;137(20):e578-e622. doi: 10.1161/CIR.0000000000000560.

4 Dai Y, Chen X, Song X, Chen X, Ma W, Lin J, Wu H, Hu X, Zhou Y, Zhang H, Liao Y, Qiu Z, Zhou Z. Immunotherapy of Endothelin-1 Receptor Type A for Pulmonary Arterial Hypertension. J Am Coll Cardiol. 2019;73(20):2567-2580. doi: 10.1016/j.jacc.2019.02.067.

5 Humbert M, Guignabert C, Bonnet S, Dorfmüller P, Klinger JR, Nicolls MR, Olschewski AJ, Pullamsetti SS, Schermuly RT, Stenmark KR, Rabinovitch M. Pathology and pathobiology of pulmonary hypertension: state of the art and research perspectives. Eur Respir J. 2019

24;53(1):1801887. doi: 10.1183/13993003.01887-2018. PMID: 30545970; PMCID: PMC6351340.

6 Buermans HP, Redout EM, Schiel AE (2005). Microarray analysis reveals pivotal divergent mRNA expression profiles early in the development of either compensated ventricular hypertrophy or heart failure. Physiol Genomics 21:314–323.

7 Drake JI, Bogaard HJ, Mizuno S (2011). Molecular signature of a right heart failure program in chronic severe pulmonary hypertension. Am J Respir Cell Mol Biol 45:1239–1247.

8 Thandavarayan RA, Chitturi KR, Guha A. Pathophysiology of Acute and Chronic Right Heart Failure. Cardiol Clin. 2020 ;38(2):149-160. doi: 10.1016/j.ccl.2020.01.009.

9 Colombo R, Siqueira R, Becker CU, Fernandes TG, Pires KM, Valença SS, Souza-rabbo MP, Araujo AS & Belló-klein A (2013). Effects of exercise on monocrotaline-induced changes in right heart function and pulmonary artery remodeling in rats. Can J Physiol Pharmacol 91, 38-44.

10 Zile MR, Baicu CF, Ikonomidis J, Stroud RE, Nietert PJ, Bradshaw AD, Slater R, Palmer BM, Buren PV, Meyer M, Redfield M, Bull D, Granzier H, & LeWinter MM (2015). Myocardial Stiffness in Patients with Heart Failure and a Preserved Ejection Fraction: Contributions of Collagen and Titin. Circulation 131, 1247-1259.

11 Schüttler D, Clauss S, Weckbach LT, Brunner S. Molecular Mechanisms of Cardiac Remodeling and Regeneration in Physical Exercise. Cells. 2019 23;8(10):1128. doi: 10.3390/cells8101128. PMID: 31547508; PMCID: PMC6829258.

12 Pacagnelli FL, Sabela AK, Okoshi K, Mariano TB, Campos DHS, Carvalho R, Cicogna AC & Vanderlei LC (2016). Preventive physical training exerts a cardioprotective effect in rats treated with monocrotaline. Int j Exp Pathol 97, 238-247.

13 Nogueira-Ferreira R, Moreira-Gon D, SilvaAna F, Duarte JA, Leite-Moreira A, Ferreira R, Henriques-Coelho T (2016). Exercise preconditioning prevents MCT-induced right ventricle remodeling through the regulation of TNF superfamily cytokines, Intern J Cardiol 203: 858–866.

14 Mariano TB, de Souza Castilho AC, de Almeida Sabela AKD, de Oliveira AC, Cury SS, Aguiar AF, et al. (2021) Preventive training does not interfere with mRNA-encoding myosin and collagen expression during pulmonary arterial hypertension. PLoS ONE 16(9): e0244768. https://doi.org/10.1371/journal.pone.0244768

15 Oshlack A, Robinson MD, Young MD. (2010) From RNA-seq reads to differential expression results. Genome Biology, 11(12): 1–10.

16 Yang IS, Kim S (2015). Analysis of Whole Transcriptome Sequencing Data: Workflow and Software. Genomics & informatics, 13(4): 119–25.

17 Clark J.D., Gebhart G.F., Gonder J.C. et al. (1997) The 1996 guide for the care and use of laboratory animals. ILAR J. 38, 41–48.

18 Gary-Bobo, G (2010). Effects of HIV protease inhibitors on progression of monocrotaline- and hypoxia-induced pulmonary hypertension in rats. Circulation 122, 1937–47.

19 Machado FB, Gobatto CA, Contartese RVL, Papoti M & Mello AR (2006). The maximal lactate steady state is ergometerdependent in experimental model using rats. Rev Bras Med Esporte 12, 259-262.

20 Carvalho JF, Masuda MO, Pompeu FAMS (2005). Method for diagnosis and control of aerobic training in rats based on lactate threshold. Comp Biochem Physiol A Mol Integr Physiol 140, 409–413.

21 Pacagnelli FL, Aguiar AF, Campos DHS, Castan EP, Souza RWAS, Almeida FLA, Carani F, Carvalho RF, Cicogna AC & Dal Pai Silva M (2016). Training improves the oxidative phenotype of muscle during the transition from cardiac hypertrophy to heart failure without altering MyoD and myogenin. Exp Physiol 101, 1075–1085.

22 Hill JA, Olson EN (2008) Plasticidade cardíaca. N Engl J Med 358: 1370-1380.

23 Rajabi M, Kassiotis C, Razeghi P, Taegtmeyer H (2007) Return to the fetal gene program protects the stressed heart: a strong hypothesis. Heart Fail Rev 12: 331–343.

24 Barry SP, Davidson SM, Townsend PA. Molecular regulation of cardiac hypertrophy. Int J Biochem Cell Biol. 2008;40(10):2023-39. doi: 10.1016/j.biocel.2008.02.020.

25 Handoko M.L., de Man F.S., Happ_e C.M. et al. (2009) Opposite effects of training in rats with table and progressive pulmonary hypertension. Circulation 120, 42–49.

26 Colombo R., Siqueira R, Conzatti A, et al. Exercise training contributes to H2O2/VEGF signaling in the lung of rats with monocrotaline-induced pulmonary hypertension. Vascul. Pharmacol. 2016; 87: 49–59.

27 Hassan MM, Hwang LY, Hatten CJ, Swaim M, Li D, Abbruzzese JL, et al. Risk factors for hepatocellular carcinoma: synergism of alcohol with viral hepatitis and diabetes mellitus. Hepatology 2002;36:1206–1213.

28 Cheng IK, Ching AK, Chan TC, Chan AW, Wong CK, Choy KW, Kwan M, Lai PB, Wong N. Reduced CRYL1 expression in hepatocellular carcinoma confers cell growth advantages and correlates with adverse patient prognosis. J Pathol. 2010 Feb;220(3):348-60. doi: 10.1002/path.2644. PMID: 19927314.

29 Li, Y.; Wang, J.; Elzo, M.A.; Gan, M.; Tang, T.; Shao, J.; Lai, T.; Ma, Y.; Jia, X.; Lai, S. Multi–Omics Analysis of Key microRNA–mRNA Metabolic Regulatory Networks in Skeletal Muscle of Obese Rabbits. Int. J. Mol. Sci. 2021, 22, 4204. https://doi.org/10.3390/ijms22084204

30 Syuhei Ishikura, Noriyuki Usami, Mayuko Araki, Akira Hara, Structural and Functional Characterization of Rabbit and Human l-Gulonate 3-Dehydrogenase, The Journal of Biochemistry, Volume 137, Issue 3, March 2005, Pages 303–314, https://doi.org/10.1093/jb/mvi033

31 Marshall JD, Bazan I, Zhang Y, Fares WH, Lee PJ. Mitochondrial dysfunction and pulmonary hypertension: cause, effect, or both. Am J Physiol Lung Cell Mol Physiol. 2018 May 1;314(5):L782-L796. doi: 10.1152/ajplung.00331.2017.

32 Hirano, Y., Ohniwa, R.L., Wada, C., Yoshimura, S.H. and Takeyasu, K. (2006), Human small G proteins, ObgH1, and ObgH2, participate in the maintenance of mitochondria and nucleolar architectures. Genes to Cells, 11: 1295-1304. https://doi.org/10.1111/j.1365-2443.2006.01017.x

33 Harvey, N.R., Voisin, S., Lea, R.A. et al. Investigating the influence of mtDNA and nuclear encoded mitochondrial variants on high intensity interval training outcomes. Sci Rep 10, 11089 (2020). https://doi.org/10.1038/s41598-020-67870-1.

34 McLellan MA, Skelly DA, Dona MSI, Squiers GT, Farrugia GE, Gaynor TL, Cohen CD, Pandey R, Diep H, Vinh A, Rosenthal NA, Pinto AR. High-Resolution Transcriptomic Profiling of the Heart During Chronic Stress Reveals Cellular Drivers of Cardiac Fibrosis and Hypertrophy. Circulation. 2020 Oct 13;142(15):1448-1463. doi: 10.1161/CIRCULATIONAHA.119.045115.

35 Revelo XS, Parthiban P, Chen C, et al. Cardiac Resident Macrophages Prevent Fibrosis and Stimulate Angiogenesis. Circ Res. 2021;129(12):1086-1101. doi:10.1161/CIRCRESAHA.121.319737

36 LIU Huiqing, JIN Fengbiao, LI Qinglian, GAO Yu, LIU Xiaoteng, HOU Ruitian. IL-34 and coronary heart disease complicated with diabetes mellitus[J]. Journal of Central South

University. Medical Science, 2021, 46(12): 1409-1414. DOI : 10.11817/j.issn.1672-7347.2021.200797

37 Mathew R, Huang J, Iacobas S, Iacobas DA. Pulmonary Hypertension Remodels the Genomic Fabrics of Major Functional Pathways. Genes (Basel). 2020;11(2):126. Published 2020 Jan 23. doi:10.3390/genes11020126

38 Wang M, Subramanian M, Abramowicz S, Murphy AJ, Gonen A, Witztum J, Welch C, Tabas I, Westerterp M, Tall AR. Interleukin-3/granulocyte macrophage colony-stimulating factor receptor promotes stem cell expansion, monocytosis, and atheroma macrophage burden in mice with hematopoietic ApoE deficiency. Arterioscler Thromb Vasc Biol. 2014;34(5):976–984. doi: 10.1161/ATVBAHA.113.303097.

39 Caillard A, Sadoune M, Cescau A, Meddour M, Gandon M, Polidano E, Delcayre C, Da Silva K, Manivet P, Gomez AM, Cohen-Solal A, Vodovar N, Li Z, Mebazaa A, Samuel JL. QSOX1, a novel actor of cardiac protection upon acute stress in mice. J Mol Cell Cardiol. 2018; 119:75-86. doi: 10.1016/j.yjmcc.2018.04.014.

40 Fraidenburg DR, Machado RF. A Review of Transcriptome Analysis in Pulmonary Vascular Diseases. Methods Mol Biol. 2018;1783:259-277. doi: 10.1007/978-1-4939-7834-2_13. PMID: 29767367; PMCID: PMC6039103.

41 Bohuslavova R, Kolar F, Kuthanova L (1985). Gene expression profiling of sex differences in HIF1- dependent adaptive cardiac responses to chronic hypoxia. J Appl Physiol 109:1195– 1202.

42 Veith C, Schermuly RT, Brandes RP (2016). Molecular mechanisms of hypoxia-inducible factorinduced pulmonary arterial smooth muscle cell alterations in pulmonary hypertension. J Physiol 594:1167–1177.

43 Martinez-Arroyo O, Selma-Soriano E, Ortega A, Cortes R, Redon J. Small Rab GTPases in Intracellular Vesicle Trafficking: The Case of Rab3A/Raphillin-3A Complex in the Kidney. Int J Mol Sci. 2021 Jul 18;22(14):7679. doi: 10.3390/ijms22147679.

ANEXO 1- NORMAS DA REVISTA BMC GENOMICS

Research article

Criteria

Research articles should report on original primary research or new experimental or computational methods, but may report on systematic reviews of published research provided they adhere to the appropriate reporting guidelines which are detailed in our editorial policies. Please note that non-commissioned pooled analyses of selected published research and bibliometric analyses will not be considered.

Data sharing

In the genomics field, there is a widely established research community expectation for data archiving in public repositories, and submission to a community-endorsed, public repository is mandatory. A list of data where deposition is required for BMC Genomics, with the appropriate repositories, can be found on the Editorial Policies Page.

We strongly encourage that all other datasets on which the conclusions of the paper rely should be available to readers. We encourage authors to ensure that their datasets are either deposited in publicly available repositories (where available and appropriate) or presented in the main manuscript or additional supporting files whenever possible. Please see Springer Nature’s information on recommended repositories.

BMC Genomics strongly supports open research, including transparency and openness in reporting. Further details of our Data availability policy can be found on the journal's About page.

Professionally produced Visual Abstracts

BMC Genomics will consider visual abstracts. As an author submitting to the journal, you may wish to make use of services provided at Springer Nature for high quality and affordable visual abstracts where you are entitled to a 20% discount. Click here to find out more about the service, and your discount will be automatically be applied when using this link.

Image integrity and standards

Cropped gels and blots can be included in the main text if it improves the clarity and conciseness of the presentation. In such cases, the cropping of the blot must be clearly evident and must be mentioned in the figure legend. Corresponding uncropped full-length gels and blot must be included in the supplementary files. These uncropped images should indicate where they were cropped, be labelled as in the main text and placed in a single supplementary figure. The manuscript's figure legends should state that 'Full-length blots/gels are presented in Supplementary Figure X'. Further information can be found under 'Digital image integrity' which are detailed on in our Standards of Reporting guidelines.

Preparing your manuscript

The information below details the section headings that you should include in your manuscript and what information should be within each section.

Please note that your manuscript must include a 'Declarations' section including all of the subheadings (please see below for more information).

Title page

The title page should:

present a title that includes, if appropriate, the study design list the full names and institutional addresses for all authors if a collaboration group should be listed as an author, please list the Group name as an author. If you would like the names of the individual members of the Group to be searchable through their individual PubMed records, please include this information in the “Acknowledgements” section in accordance with the instructions below

indicate the corresponding author

Abstract

The Abstract should not exceed 350 words. Please minimize the use of abbreviations and do not cite references in the abstract. The abstract must include the following separate sections:

Background: the context and purpose of the study

Results: the main findings

Conclusions: a brief summary and potential implications

Keywords

Three to ten keywords representing the main content of the article.

Background

The Background section should explain the background to the study, its aims, a summary of the existing literature and why this study was necessary.

Results

This should include the findings of the study including, if appropriate, results of statistical analysis which must be included either in the text or as tables and figures.

Discussion

For research articles this section should discuss the implications of the findings in context of existing research and highlight limitations of the study. For study protocols and methodology manuscripts this section should include a discussion of any practical or operational issues involved in performing the study and any issues not covered in other sections.

Conclusions

This should state clearly the main conclusions and provide an explanation of the importance and relevance of the study to the field.

Methods

The methods section should include:

the aim, design and setting of the study the characteristics of participants or description of materials a clear description of all processes, interventions and comparisons. Generic names should generally be used. When proprietary brands are used in research, include the brand names in parentheses the type of statistical analysis used, including a power calculation if appropriate List of abbreviations If abbreviations are used in the text they should be defined in the text at first use, and a list of abbreviations can be provided.

Declarations

All manuscripts must contain the following sections under the heading 'Declarations': Ethics approval and consent to participate

Consent for publication

Availability of data and materials

Competing interests

Funding

Authors' contributions

Acknowledgements

Authors' information (optional)

Please see below for details on the information to be included in these sections.

If any of the sections are not relevant to your manuscript, please include the heading and write 'Not applicable' for that section.

Ethics approval and consent to participate

Manuscripts reporting studies involving human participants, human data or human tissue must:

include a statement on ethics approval and consent (even where the need for approval was waived) include the name of the ethics committee that approved the study and the committee’s reference number if appropriate

Studies involving animals must include a statement on ethics approval and for experimental studies involving client-owned animals, authors must also include a statement on informed consent from the client or owner.

See our editorial policies for more information.

If your manuscript does not report on or involve the use of any animal or human data or tissue, please state “Not applicable” in this section.

Consent for publication

If your manuscript contains any individual person’s data in any form (including any individual details, images or videos), consent for publication must be obtained from that person, or in

the case of children, their parent or legal guardian. All presentations of case reports must have consent for publication.

You can use your institutional consent form or our consent form if you prefer. You should not send the form to us on submission, but we may request to see a copy at any stage (including after publication).

See our editorial policies for more information on consent for publication.

If your manuscript does not contain data from any individual person, please state “Not applicable” in this section.

Availability of data and materials

All manuscripts must include an ‘Availability of data and materials’ statement. Data availability statements should include information on where data supporting the results reported in the article can be found including, where applicable, hyperlinks to publicly archived datasets analysed or generated during the study. By data we mean the minimal dataset that would be necessary to interpret, replicate and build upon the findings reported in the article. We recognise it is not always possible to share research data publicly, for instance when individual privacy could be compromised, and in such instances data availability should still be stated in the manuscript along with any conditions for access.

Data availability statements can take one of the following forms (or a combination of more than one if required for multiple datasets):

The datasets generated and/or analysed during the current study are available in the [NAME] repository, [PERSISTENT WEB LINK TO DATASETS]

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

All data generated or analysed during this study are included in this published article [and its supplementary information files].

The datasets generated and/or analysed during the current study are not publicly available due [REASON WHY DATA ARE NOT PUBLIC] but are available from the corresponding author on reasonable request.

Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

The data that support the findings of this study are available from [third party name] but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of [third party name].

Not applicable. If your manuscript does not contain any data, please state 'Not applicable' in this section.

More examples of template data availability statements, which include examples of openly available and restricted access datasets, are available here.

BioMed Central also requires that authors cite any publicly available data on which the conclusions of the paper rely in the manuscript. Data citations should include a persistent identifier (such as a DOI) and should ideally be included in the reference list. Citations of datasets, when they appear in the reference list, should include the minimum information recommended by DataCite and follow journal style. Dataset identifiers including DOIs should be expressed as full URLs. For example:

Hao Z, AghaKouchak A, Nakhjiri N, Farahmand A. Global integrated drought monitoring and prediction system (GIDMaPS) data sets. figshare. 2014. http://dx.doi.org/10.6084/m9.figshare.853801

With the corresponding text in the Availability of data and materials statement:

The datasets generated during and/or analysed during the current study are available in the [NAME] repository, [PERSISTENT WEB LINK TO DATASETS].[Reference number]

If you wish to co-submit a data note describing your data to be published in BMC Research Notes, you can do so by visiting our submission portal. Data notes support open data and help authors to comply with funder policies on data sharing. Co-published data notes will be linked to the research article the data support (example).

Competing interests

All financial and non-financial competing interests must be declared in this section.

See our editorial policies for a full explanation of competing interests. If you are unsure whether you or any of your co-authors have a competing interest please contact the editorial office.

Please use the authors initials to refer to each authors' competing interests in this section.

If you do not have any competing interests, please state "The authors declare that they have no competing interests" in this section.

Funding

All sources of funding for the research reported should be declared. The role of the funding body in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript should be declared.

Authors' contributions

The individual contributions of authors to the manuscript should be specified in this section. Guidance and criteria for authorship can be found in our editorial policies.

Please use initials to refer to each author's contribution in this section, for example: "FC analyzed and interpreted the patient data regarding the hematological disease and the transplant. RH performed the histological examination of the kidney, and was a major contributor in writing the manuscript. All authors read and approved the final manuscript."

Acknowledgements

Please acknowledge anyone who contributed towards the article who does not meet the criteria for authorship including anyone who provided professional writing services or materials.

Authors should obtain permission to acknowledge from all those mentioned in the Acknowledgements section.

See our editorial policies for a full explanation of acknowledgements and authorship criteria.

If you do not have anyone to acknowledge, please write "Not applicable" in this section.

Group authorship (for manuscripts involving a collaboration group): if you would like the names of the individual members of a collaboration Group to be searchable through their individual PubMed records, please ensure that the title of the collaboration Group is included on the title page and in the submission system and also include collaborating author names as the last paragraph of the “Acknowledgements” section. Please add authors in the format First Name, Middle initial(s) (optional), Last Name. You can add institution or country information for each author if you wish, but this should be consistent across all authors.

Please note that individual names may not be present in the PubMed record at the time a published article is initially included in PubMed as it takes PubMed additional time to code this information.

Authors' information

This section is optional.

You may choose to use this section to include any relevant information about the author(s) that may aid the reader's interpretation of the article, and understand the standpoint of the author(s). This may include details about the authors' qualifications, current positions they hold at institutions or societies, or any other relevant background information. Please refer to authors using their initials. Note this section should not be used to describe any competing interests.

Footnotes

Footnotes can be used to give additional information, which may include the citation of a reference included in the reference list. They should not consist solely of a reference citation, and they should never include the bibliographic details of a reference. They should also not contain any figures or tables.

Footnotes to the text are numbered consecutively; those to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data). Footnotes to the title or the authors of the article are not given reference symbols.

Always use footnotes instead of endnotes.

References

Examples of the Vancouver reference style are shown below.

See our editorial policies for author guidance on good citation practice

Web links and URLs: All web links and URLs, including links to the authors' own websites, should be given a reference number and included in the reference list rather than within the text of the manuscript. They should be provided in full, including both the title of the site and the URL, as well as the date the site was accessed, in the following format: The Mouse Tumor Biology Database. http://tumor.informatics.jax.org/mtbwi/index.do. Accessed 20 May 2013. If an author or group of authors can clearly be associated with a web link, such as for weblogs, then they should be included in the reference.

Example reference style:

Article within a journal

Smith JJ. The world of science. Am J Sci. 1999;36:234-5.

Article within a journal (no page numbers)

Rohrmann S, Overvad K, Bueno-de-Mesquita HB, Jakobsen MU, Egeberg R, Tjønneland A, et al. Meat consumption and mortality - results from the European Prospective Investigation into Cancer and Nutrition. BMC Medicine. 2013;11:63.

Article within a journal by DOI

Slifka MK, Whitton JL. Clinical implications of dysregulated cytokine production. Dig J Mol Med. 2000; doi:10.1007/s801090000086.

Article within a journal supplement

Frumin AM, Nussbaum J, Esposito M. Functional asplenia: demonstration of splenic activity by bone marrow scan. Blood 1979;59 Suppl 1:26-32.

Book chapter, or an article within a book

Wyllie AH, Kerr JFR, Currie AR. Cell death: the significance of apoptosis. In: Bourne GH, Danielli JF, Jeon KW, editors. International review of cytology. London: Academic; 1980. p. 251-306.

OnlineFirst chapter in a series (without a volume designation but with a DOI)

Saito Y, Hyuga H. Rate equation approaches to amplification of enantiomeric excess and chiral symmetry breaking. Top Curr Chem. 2007. doi:10.1007/128_2006_108.

Complete book, authored

Blenkinsopp A, Paxton P. Symptoms in the pharmacy: a guide to the management of common illness. 3rd ed. Oxford: Blackwell Science; 1998.

Online document

Doe J. Title of subordinate document. In: The dictionary of substances and their effects. Royal Society of Chemistry. 1999. http://www.rsc.org/dose/title of subordinate document. Accessed 15 Jan 1999.

Online database

Healthwise Knowledgebase. US Pharmacopeia, Rockville. 1998. http://www.healthwise.org. Accessed 21 Sept 1998.

Supplementary material/private homepage

Doe J. Title of supplementary material. 2000. http://www.privatehomepage.com. Accessed 22 Feb 2000.

University site

Doe, J: Title of preprint. http://www.uni-heidelberg.de/mydata.html (1999). Accessed 25 Dec 1999.

FTP site

Doe, J: Trivial HTTP, RFC2169. ftp://ftp.isi.edu/in-notes/rfc2169.txt (1999). Accessed 12 Nov 1999.

Organization site

ISSN International Centre: The ISSN register. http://www.issn.org (2006). Accessed 20 Feb 2007.

Dataset with persistent identifier

Zheng L-Y, Guo X-S, He B, Sun L-J, Peng Y, Dong S-S, et al. Genome data from sweet and grain sorghum (Sorghum bicolor). GigaScience Database. 2011. http://dx.doi.org/10.5524/100012.

Figures, tables and additional files

See General formatting guidelines for information on how to format figures, tables and additional files.

Preparing your manuscript

This section provides general style and formatting information only. Formatting guidelines for specific article types can be found below.

Research article

Database article

Software article

Review

General formatting guidelines

Preparing main manuscript text

Preparing illustrations and figures

Preparing tables

Preparing additional files

Preparing figures

Back to top

When preparing figures, please follow the formatting instructions below.

Figures should be numbered in the order they are first mentioned in the text, and uploaded in this order. Multi-panel figures (those with parts a, b, c, d etc.) should be submitted as a single composite file that contains all parts of the figure.

Figures should be uploaded in the correct orientation.

Figure titles (max 15 words) and legends (max 300 words) should be provided in the main manuscript, not in the graphic file.

Figure keys should be incorporated into the graphic, not into the legend of the figure.

Each figure should be closely cropped to minimize the amount of white space surrounding the illustration. Cropping figures improves accuracy when placing the figure in combination with other elements when the accepted manuscript is prepared for publication on our site. For more information on individual figure file formats, see our detailed instructions.

Individual figure files should not exceed 10 MB. If a suitable format is chosen, this file size is adequate for extremely high quality figures.

Please note that it is the responsibility of the author(s) to obtain permission from the copyright holder to reproduce figures (or tables) that have previously been published elsewhere. In order for all figures to be open access, authors must have permission from the rights holder if they wish to include images that have been published elsewhere in non open access journals. Permission should be indicated in the figure legend, and the original source included in the reference list.

Figure file types

We accept the following file formats for figures:

EPS (suitable for diagrams and/or images)

PDF (suitable for diagrams and/or images)

Microsoft Word (suitable for diagrams and/or images, figures must be a single page)

PowerPoint (suitable for diagrams and/or images, figures must be a single page)

TIFF (suitable for images)

JPEG (suitable for photographic images, less suitable for graphical images)

PNG (suitable for images)

BMP (suitable for images)

CDX (ChemDraw - suitable for molecular structures)

For information and suggestions of suitable file formats for specific figure types, please see our author academy.

Figure size and resolution

Figures are resized during publication of the final full text and PDF versions to conform to the BioMed Central standard dimensions, which are detailed below.

Figures on the web:

width of 600 pixels (standard), 1200 pixels (high resolution).

Figures in the final PDF version:
width of 85 mm for half page width figure
width of 170 mm for full page width figure
maximum height of 225 mm for figure and legend
image resolution of approximately 300 dpi (dots per inch) at the final size
Figures should be designed such that all information, including text, is legible at these dimensions. All lines should be wider than 0.25 pt when constrained to standard figure widths. All fonts must be embedded.
Figure file compression
Vector figures should if possible be submitted as PDF files, which are usually more compact than EPS files.
TIFF files should be saved with LZW compression, which is lossless (decreases file size without decreasing quality) in order to minimize upload time.
JPEG files should be saved at maximum quality.
Conversion of images between file types (especially lossy formats such as JPEG) should be kept to a minimum to avoid degradation of quality.
If you have any questions or are experiencing a problem with figures, please contact the customer service team at info@biomedcentral.com.
Preparing main manuscript text
Back to top
Quick points:
Use double line spacing
Include line and page numbering
Use SI units: Please ensure that all special characters used are embedded in the text, otherwise they will be lost during conversion to PDF
Do not use page breaks in your manuscript
File formats
The following word processor file formats are acceptable for the main manuscript document:
Microsoft word (DOC, DOCX)
Rich text format (RTF)
TeX/LaTeX (use BioMed Central's TeX template)
Please note: editable files are required for processing in production. If your manuscript contains any non-editable files (such as PDFs) you will be required to re-submit an editable file when you submit your revised manuscript, or after editorial acceptance in case no revision is necessary.
Additional information for TeX/LaTeX users

Please use BioMed Central's TeX template and BibTeX stylefile if you use TeX format. Submit your references using either a bib or bbl file. When submitting TeX submissions, please submit both your TeX file and your bib/bbl file as manuscript files. Please also convert your TeX file into a PDF (please do not use a DIV file) and submit this PDF as a supplementary file with the name 'Reference PDF'. This PDF will be used by our production team as a reference point to check the layout of the article as the author intended.

The Editorial Manager system checks for any errors in the Tex files. If an error is present then the system PDF will display LaTex code and highlight and explain the error in a section beginning with an exclamation mark (!).

All relevant editable source files must be uploaded during the submission process. Failing to submit these source files will cause unnecessary delays in the production process.

TeX templates

BioMedCentral_article (ZIP format) - preferred template

article (part of the standard TeX distribution)

amsart (part of the standard TeX distribution)

Style and language

For editors and reviewers to accurately assess the work presented in your manuscript you need to ensure the English language is of sufficient quality to be understood. If you need help with writing in English you should consider:

Getting a fast, free online grammar check.

Visiting the English language tutorial which covers the common mistakes when writing in English.

Asking a colleague who is proficient in English to review your manuscript for clarity.

Using a professional language editing service where editors will improve the English to ensure that your meaning is clear and identify problems that require your review. Two such services are provided by our affiliates Nature Research Editing Service and American Journal Experts. BMC authors are entitled to a 10% discount on their first submission to either of these services. To claim 10% off English editing from Nature Research Editing Service, click here. To claim 10% off American Journal Experts, click here.

Please note that the use of a language editing service is not a requirement for publication in the journal and does not imply or guarantee that the article will be selected for peer review or accepted.

Data and materials

For all journals, BioMed Central strongly encourages all datasets on which the conclusions of the manuscript rely to be either deposited in publicly available repositories (where available and appropriate) or presented in the main paper or additional supporting files, in machine-

readable format (such as spread sheets rather than PDFs) whenever possible. Please see the list of recommended repositories in our editorial policies.

For some journals, deposition of the data on which the conclusions of the manuscript rely is an absolute requirement. Please check the Instructions for Authors for the relevant journal and article type for journal specific policies.

For all manuscripts, information about data availability should be detailed in an ‘Availability of data and materials’ section. For more information on the content of this section, please see the Declarations section of the relevant journal’s Instruction for Authors. For more information on BioMed Centrals policies on data availability, please see our [editorial policies].

Formatting the 'Availability of data and materials' section of your manuscript

The following format for the 'Availability of data and materials section of your manuscript should be used:

"The dataset(s) supporting the conclusions of this article is(are) available in the [repository name] repository, [unique persistent identifier and hyperlink to dataset(s) in http:// format]."

The following format is required when data are included as additional files:

"The dataset(s) supporting the conclusions of this article is(are) included within the article (and its additional file(s))."

BioMed Central endorses the Force 11 Data Citation Principles and requires that all publicly available datasets be fully referenced in the reference list with an accession number or unique identifier such as a DOI.

For databases, this section should state the web/ftp address at which the database is available and any restrictions to its use by non-academics.

For software, this section should include:

Project name: e.g. My bioinformatics project

Project home page: e.g. http://sourceforge.net/projects/mged

Archived version: DOI or unique identifier of archived software or code in repository (e.g. enodo)

Operating system(s): e.g. Platform independent

Programming language: e.g. Java

Other requirements: e.g. Java 1.3.1 or higher, Tomcat 4.0 or higher

License: e.g. GNU GPL, FreeBSD etc.

Any restrictions to use by non-academics: e.g. licence needed

Information on available repositories for other types of scientific data, including clinical data, can be found in our editorial policies.

References

See our editorial policies for author guidance on good citation practice.

Please check the submission guidelines for the relevant journal and article type.

What should be cited?

Only articles, clinical trial registration records and abstracts that have been published or are in press, or are available through public e-print/preprint servers, may be cited.

Unpublished abstracts, unpublished data and personal communications should not be included in the reference list, but may be included in the text and referred to as "unpublished observations" or "personal communications" giving the names of the involved researchers. Obtaining permission to quote personal communications and unpublished data from the cited colleagues is the responsibility of the author. Only footnotes are permitted. Journal abbreviations follow Index Medicus/MEDLINE.

Any in press articles cited within the references and necessary for the reviewers' assessment of the manuscript should be made available if requested by the editorial office.

How to format your references

Please check the Instructions for Authors for the relevant journal and article type for examples of the relevant reference style.

Web links and URLs: All web links and URLs, including links to the authors' own websites, should be given a reference number and included in the reference list rather than within the text of the manuscript. They should be provided in full, including both the title of the site and the URL, as well as the date the site was accessed, in the following format: The Mouse Tumor Biology Database. http://tumor.informatics.jax.org/mtbwi/index.do. Accessed 20 May 2013. If an author or group of authors can clearly be associated with a web link, such as for weblogs, then they should be included in the reference.

Authors may wish to make use of reference management software to ensure that reference lists are correctly formatted.

Preparing tables

Back to top

When preparing tables, please follow the formatting instructions below.

Tables should be numbered and cited in the text in sequence using Arabic numerals (i.e. Table 1, Table 2 etc.).

Tables less than one A4 or Letter page in length can be placed in the appropriate location within the manuscript.

Tables larger than one A4 or Letter page in length can be placed at the end of the document text file. Please cite and indicate where the table should appear at the relevant location in the text file so that the table can be added in the correct place during production.

Larger datasets, or tables too wide for A4 or Letter landscape page can be uploaded as additional files. Please see [below] for more information.

Tabular data provided as additional files can be uploaded as an Excel spreadsheet (.xls) or comma separated values (.csv). Please use the standard file extensions.

Table titles (max 15 words) should be included above the table, and legends (max 300 words) should be included underneath the table.
Tables should not be embedded as figures or spreadsheet files, but should be formatted using ‘Table object’ function in your word processing program.
Color and shading may not be used. Parts of the table can be highlighted using superscript, numbering, lettering, symbols or bold text, the meaning of which should be explained in a table legend.
Commas should not be used to indicate numerical values.
If you have any questions or are experiencing a problem with tables, please contact the customer service team at info@biomedcentral.com.
Preparing additional files
Back to top
As the length and quantity of data is not restricted for many article types, authors can provide datasets, tables, movies, or other information as additional files.
All Additional files will be published along with the accepted article. Do not include files such as patient consent forms, certificates of language editing, or revised versions of the main manuscript document with tracked changes. Such files, if requested, should be sent by email to the journal’s editorial email address, quoting the manuscript reference number. Please do not send completed patient consent forms unless requested.
Results that would otherwise be indicated as "data not shown" should be included as additional files. Since many web links and URLs rapidly become broken, BioMed Central requires that supporting data are included as additional files, or deposited in a recognized repository. Please do not link to data on a personal/departmental website. Do not include any individual participant details. The maximum file size for additional files is 20 MB each, and files will be virus-scanned on submission. Each additional file should be cited in sequence within the main body of text.

If additional material is provided, please list the following information in a separate section of the manuscript text:
File name (e.g. Additional file 1)
File format including the correct file extension for example .pdf, .xls, .txt, .pptx (including name and a URL of an appropriate viewer if format is unusual)
Title of data
Description of data
Additional files should be named "Additional file 1" and so on and should be referenced explicitly by file name within the body of the article, e.g. 'An additional movie file shows this in more detail [see Additional file 1]'.

For further guidance on how to use Additional files or recommendations on how to present particular types of data or information, please see How to use additional files.

ANEXO 2 - LISTA DE GENES ALTERADOS

Retsat	retinol saturase (all trans retinol 13,14 reductase)
LOC102550650	uncharacterized LOC102550650
LOC102546505	uncharacterized LOC102546505
LOC102553405	protein NYNRIN-like
Clec1b	C-type lectin domain family 1, member B
Pbld1	phenazine biosynthesis-like protein domain containing 1
Pcyt2	phosphate cytidylyltransferase 2, ethanolamine
RT1-Da	RT1 class II, locus Da
LOC102555587	uncharacterized LOC102555587
Gzma	granzyme A
Oxr1	oxidation resistance 1
LOC102554994	protein NYNRIN-like
Bre	brain and reproductive organ-expressed (TNFRSF1A modulator)
Acta1	actin, alpha 1, skeletal muscle
Gypa	glycophorin A
Psmb6	proteasome subunit beta 6
LOC100360846	INTERACTS WITH (S)-nicotine (ortholog) AND 2-naphthylamine (ortholog) AND benzo[a]pyrene (ortholog)
Bdh1	3-hydroxybutyrate dehydrogenase, type 1
Acta2	actin, alpha 2, smooth muscle, aorta
Trim54	tripartite motif-containing 54
Lingo4; Rorc	leucine rich repeat and Ig domain containing 4; RAR-related orphan receptor C
Susd2	sushi domain containing 2
Sh2d3c	SH2 domain containing 3C
Obfc1	oligonucleotide/oligosaccharide-binding fold containing 1
Esam	endothelial cell adhesion molecule
Tagln	transgelin
Mrps18b	mitochondrial ribosomal protein S18B
Eif2a	eukaryotic translation initiation factor 2A; ENCODES a protein that exhibits ribosome binding (ortholog) AND translation initiation factor activity (ortholog) AND tRNA binding (ortholog) AND INVOLVED IN positive regulation of signal transduction (ortholog)

	AND protein phosphorylation (ortholog) AND regulation of translation (ortholog) AND PARTICIPATES IN ceramide signaling pathway AND transforming growth factor-beta Smad dependent signaling pathway AND FOUND IN blood microparticle (ortholog) AND cytoplasm (ortholog) AND eukaryotic translation initiation factor 2 complex (ortholog) AND INTERACTS WITH 1 4-dithiothreitol AND 2 3 7 8-tetrachlorodibenzodioxine AND 2 6-dinitrotoluene; eukaryotic translation initiation factor 2A [Source:RefSeq peptide;Acc:NP_001102809]
Hbb-b1; LOC689064	hemoglobin, beta adult major chain [Source:RGD Symbol;Acc:1595848]; beta-globinhemoglobin, beta adult major chain; ENCODES a protein that exhibits heme binding (inferred) AND iron ion binding (inferred) AND oxygen binding (inferred) AND INVOLVED IN oxygen transport (inferred) AND PARTICIPATES IN Plasmodium infection pathway AND Trypanosoma brucei infection pathway AND FOUND IN hemoglobin complex (inferred) AND INTERACTS WITH (-)-demecolcine (ortholog) AND 17beta-estradiol (ortholog) AND 2 2' 4 4' 5 5'-hexachlorobiphenyl (ortholog); hemoglobin, beta adult major chain (Hbb-b1), mRNA,
Gpr22	G protein-coupled receptor 22
Cd1d1	CD1d1 molecule
Adh1	alcohol dehydrogenase 1 (class I)
Tuba1a	tubulin, alpha 1A
Rpl36	ribosomal protein L36
Mcam	melanoma cell adhesion molecule
Fis1	fission, mitochondrial 1
LOC100361060	ribosomal protein L36-like [Source:RGD Symbol;Acc:2320900]; ENCODES a protein that exhibits structural constituent of ribosome (inferred) AND INVOLVED IN translation (inferred) AND FOUND IN ribosome (inferred) AND INTERACTS WITH 17beta-estradiol (ortholog) AND cisplatin (ortholog) AND cyclosporin A (ortholog)
Hspb6	heat shock protein, alpha-crystallin-related, B6
Trmt10c	tRNA methyltransferase 10 homolog C (S, cerevisiae)
Cd226	CD226 molecule
Hbb-b1	hemoglobin, beta adult major chain

LOC689064	beta-globin
Smpdl3a	sphingomyelin phosphodiesterase, acid-like 3A
Tbccd1	TBCC domain containing 1
Dpep2	dipeptidase 2
Zfp605; AABR07036649,2	zinc finger protein 605 [Source:RGD Symbol;Acc:2318406]; -
Hmgn5b	high mobility group nucleosome binding domain 5B
LOC100362814	hypothetical protein LOC100362814 [Source:RGD Symbol;Acc:2323964]; INTERACTS WITH 17beta-estradiol (ortholog) AND doxorubicin (ortholog) AND valproic acid (ortholog)
Scn1a	sodium channel, voltage-gated, type I, alpha subunit
LOC100359616	60S ribosomal protein L36; ENCODES a protein that exhibits structural constituent of ribosome (inferred) AND INVOLVED IN translation (inferred) AND FOUND IN ribosome (inferred) AND INTERACTS WITH 17beta-estradiol (ortholog) AND cisplatin (ortholog) AND cyclosporin A (ortholog)
Stxbp6	syntaxin binding protein 6 (amisyn)
Elk3	ELK3, member of ETS oncogene family
Ccnd1	cyclin D1
S100a9	S100 calcium binding protein A9
Tuba4a	tubulin, alpha 4A
LOC100911813; LOC102550397	sperm motility kinase X-like; zinc finger protein 709-like
Itgb1bp2	integrin beta 1 binding protein 2
LOC689064	beta-globin (LOC689064), mRNA; ENCODES a protein that exhibits heme binding (inferred) AND iron ion binding (inferred) AND oxygen binding (inferred) AND INVOLVED IN oxygen transport (inferred) AND ASSOCIATED WITH Diabetes Mellitus Experimental AND FOUND IN hemoglobin complex (inferred) AND INTERACTS WITH (-)-demecolcine (ortholog) AND 17beta-estradiol (ortholog) AND 2 2' 4 4' 5 5'-hexachlorobiphenyl (ortholog)
Cpxm2	carboxypeptidase X (M14 family), member 2
Hbb	hemoglobin, beta
Decr1	2,4-dienoyl CoA reductase 1, mitochondrial

Smtn	smoothelin
LOC102553193	zinc finger protein 120-like
Ppbp	pro-platelet basic protein (chemokine (C-X-C motif) ligand 7)
Ccl6	chemokine (C-C motif) ligand 6
Dbp	D site of albumin promoter (albumin D-box) binding protein
Tspan7	tetraspanin 7
Agr2	anterior gradient 2, protein disulphide isomerase family member
Snrk	SNF related kinase
Lztfl1	leucine zipper transcription factor-like 1
Ncs1	neuronal calcium sensor 1
Stab1	stabilin 1 [Source:RGD Symbol;Acc:2324745]; ENCODES a protein that exhibits low-density lipoprotein particle binding (ortholog) AND low-density lipoprotein receptor activity (ortholog) AND scavenger receptor activity (ortholog) AND INVOLVED IN cell-cell signaling (ortholog) AND defense response to bacterium (ortholog) AND negative regulation of angiogenesis (ortholog) AND FOUND IN integral component of plasma membrane (ortholog) AND INTERACTS WITH 17beta-estradiol (ortholog) AND 2 3 7 8-tetrachlorodibenzodioxine (ortholog) AND 2-butoxyethanol (ortholog)
Dnajb9	DnaJ (Hsp40) homolog, subfamily B, member 9
Npy; LOC100912228	neuropeptide Y (Npy), mRNA; pro-neuropeptide Y-like; ENCODES a protein that exhibits hormone activity (inferred) AND FOUND IN extracellular region (inferred) AND INTERACTS WITH (R)-noradrenaline (ortholog) AND 5-aza-2'-deoxycytidine (ortholog) AND all-trans-retinoic acid (ortholog)
LOC100363171	histone variant H2al2-like
Ly6e	lymphocyte antigen 6 complex, locus E
LOC689840	LRRGT00142
Plac8	placenta-specific 8
Cep19	centrosomal protein 19
Mgst1	microsomal glutathione S-transferase 1
Xpnpep2; LOC102551432	X-prolyl aminopeptidase (aminopeptidase P) 2, membrane-bound; ENCODES a protein that exhibits aminopeptidase activity (inferred) AND metal ion binding (inferred) AND INVOLVED IN proteolysis (inferred) AND PARTICIPATES IN kallikrein-kinin cascade

	pathway AND FOUND IN extracellular vesicular exosome (ortholog) AND INTERACTS WITH 2 3 7 8-tetrachlorodibenzodioxine AND 2 4-dinitrotoluene AND 2 6-dinitrotoluene; xaa-Pro aminopeptidase 2-like
Npy	neuropeptide Y
Fancl	Fanconi anemia, complementation group L
LOC100134871	beta globin minor gene
Pdgfra	platelet derived growth factor receptor, alpha polypeptide
Zfp804a	zinc finger protein 804A
Actg2	actin, gamma 2, smooth muscle, enteric
Hmg20a	high mobility group 20A
Lcn2	lipocalin 2
Il34	interleukin 34
Id1	inhibitor of DNA binding 1
Oat	ornithine aminotransferase
Lgals3bp	lectin, galactoside-binding, soluble, 3 binding protein
Cmtm8	CKLF-like MARVEL transmembrane domain containing 8
Per3	period circadian clock 3
Fads1	fatty acid desaturase 1
LOC100360439	ribosomal protein L36-like [Source:RGD Symbol;Acc:2322117]; ENCODES a protein that exhibits structural constituent of ribosome (inferred) AND INVOLVED IN translation (inferred) AND FOUND IN cytosolic large ribosomal subunit (inferred) AND nucleolus (inferred) AND INTERACTS WITH 17beta-estradiol (ortholog) AND cisplatin (ortholog) AND cyclosporin A (ortholog)
Fundc2	FUN14 domain containing 2
Mrgprf	MAS-related GPR, member F
Bhlhe40	basic helix-loop-helix family, member e40
Acvrl1	activin A receptor type II-like 1
Slc26a10	solute carrier family 26, member 10
Sts	steroid sulfatase (microsomal), isozyme S
Tmem88	transmembrane protein 88
Rgs7bp	regulator of G-protein signaling 7 binding protein
RGD1566325	Uncharacterized protein; similar to regulator of sex-limitation candidate 16
Wwp2	WW domain containing E3 ubiquitin protein ligase 2

Spryd7	SPRY domain containing 7
Perp	PERP, TP53 apoptosis effector
LOC102553917	putative zinc finger protein 724-like
Apip	APAF1 interacting protein
Sorl1	sortilin-related receptor, LDLR class A repeats-containing
Chit1	chitinase 1 (chitotriosidase)
Olr639	olfactory receptor 639
Olr639; Olr636	olfactory receptor 639 (Olr639), mRNA; ENCODES a protein that exhibits olfactory receptor activity (inferred) AND INVOLVED IN detection of chemical stimulus involved in sensory perception of smell (inferred) AND G-protein coupled receptor signaling pathway (inferred) AND FOUND IN integral component of membrane (inferred)
Olr639; LOC100910858	olfactory receptor 639 (Olr639), mRNA; olfactory receptor 4P4-like
Angpt1	angiopoietin 1
Olr625	olfactory receptor 625
Setbp1	SET binding protein 1
Asnsd1	asparagine synthetase domain containing 1
Zfp654; snalor	zinc finger protein 654 [Source:RGD Symbol;Acc:1308321]; ENCODES a protein that exhibits metal ion binding (inferred) AND INTERACTS WITH 2 3 7 8-tetrachlorodibenzodioxine AND benzo[a]pyrene (ortholog) AND copper atom (ortholog); Chalmel, et, al, AceView Annotation snalor,aSep08; zinc finger protein 654
Unc5c	unc-5 netrin receptor C
Ttk	Ttk protein kinase
Cidea	cell death-inducing DFFA-like effector a
Supv3l1	SUV3-like helicase
Fam58b	family with sequence similarity 58, member B
Wwox	WW domain-containing oxidoreductase
Ndufv3	NADH dehydrogenase (ubiquinone) flavoprotein 3
Trim72	tripartite motif containing 72, E3 ubiquitin protein ligase
Csrp1	cysteine and glycine-rich protein 1
Gsta4	glutathione S-transferase alpha 4
Hey1	hes-related family bHLH transcription factor with YRPW motif 1
Gpr137	G protein-coupled receptor 137

Drosha	drosha, ribonuclease type III
Rrbp1	ribosome binding protein 1
Ckap2	cytoskeleton associated protein 2
LOC102554183	uncharacterized LOC102554183
Pomp	PARTICIPATES IN ubiquitin/proteasome degradation pathway AND FOUND IN proteasome complex (inferred) AND INTERACTS WITH copper(2+) sulfate (ortholog) AND copper(II) chloride (ortholog) AND cyclosporin A (ortholog); proteasome maturation protein; proteasome maturation protein (Pomp), mRNA,
Gzmk	granzyme K
Hpd	4-hydroxyphenylpyruvate dioxygenase
Mansc1	MANSC domain containing 1; MANSC domain containing 1hypothetical protein LOC690615; INTERACTS WITH 17beta-estradiol (ortholog) AND 2 3 7 8-tetrachlorodibenzodioxine (ortholog) AND L-methionine (ortholog)
Lgals5	lectin, galactose binding, soluble 5
Mettl18	methyltransferase like 18
Cog5; zertu	INVOLVED IN intra-Golgi vesicle-mediated transport (inferred) AND ASSOCIATED WITH CONGENITAL DISORDER OF GLYCOSYLATION TYPE IIi (ortholog) AND FOUND IN Golgi apparatus (ortholog) AND Golgi transport complex (ortholog) AND membrane (ortholog) AND INTERACTS WITH benzo[a]pyrene (ortholog) AND copper(2+) sulfate (ortholog) AND coumestrol (ortholog); Chalmel, et, al, AceView Annotation zertu,aSep08
LOC100909441	FOUND IN cytoplasmic microtubule (ortholog) AND nucleus (ortholog) AND INTERACTS WITH 2 3 7 8-tetrachlorodibenzodioxine AND bis(2-ethylhexyl) phthalate AND ketamine
Pqlc1	PQ loop repeat containing 1
Ndufv3-ps1	NADH dehydrogenase (ubiquinone) flavoprotein 3, pseudogene 1 [Source:RGD Symbol;Acc:1583292]; NADH dehydrogenase (ubiquinone) flavoprotein 3, pseudogene 1
Tmem97	transmembrane protein 97
MGC108823	similar to interferon-inducible GTPase; ENCODES a protein that exhibits GTP binding (inferred) AND hydrolase activity acting on acid anhydrides (inferred) AND INVOLVED IN metabolic process

	(inferred) AND FOUND IN membrane (inferred); similar to interferon-inducible GTPase (MGC108823), mRNA,
Kbtbd12; LOC502859	Protein LOC502859; null
Yif1a	Yip1 interacting factor homolog A (S, cerevisiae)
Nars2	asparaginyl-tRNA synthetase 2 (mitochondrial)(putative)
Vsig10	V-set and immunoglobulin domain containing 10
Acss3	acyl-CoA synthetase short-chain family member 3
Fscn1	fascin actin-bundling protein 1
Pglyrp3	peptidoglycan recognition protein 3
Csgalnact1	chondroitin sulfate N-acetylgalactosaminyltransferase 1
Rnf26	ring finger protein 26
Fmnl2; RGD1560248	Protein Fmnl2; ENCODES a protein that exhibits actin binding (inferred) AND Rho GTPase binding (inferred) AND INVOLVED IN cortical actin cytoskeleton organization (ortholog) AND cytoskeleton organization (ortholog) AND regulation of cell morphogenesis (ortholog) AND INTERACTS WITH thioacetamide AND (-)-epigallocatechin 3-gallate (ortholog) AND 17beta-estradiol (ortholog); similar to formin-like 2 isoform B
Tp53i11	tumor protein p53 inducible protein 11
Tie1	tyrosine kinase with immunoglobulin-like and EGF-like domains 1
Hk2	hexokinase 2
Mreg	melanoregulin
Hspa13	heat shock protein 70 family, member 13
Ehd1	EH-domain containing 1
LOC100359498; LOC100359825; Rpl35a	ribosomal protein L35a-like; ENCODES a protein that exhibits poly(A) RNA binding (ortholog) AND INVOLVED IN ribosomal large subunit biogenesis (ortholog) AND rRNA processing (ortholog) AND ASSOCIATED WITH Diamond-Blackfan Anemia 5 (ortholog) AND FOUND IN cytosolic large ribosomal subunit (ortholog) AND extracellular vesicular exosome (ortholog) AND membrane (ortholog) AND INTERACTS WITH 2 3 7 8-tetrachlorodibenzodioxine AND 2 6-dinitrotoluene AND ammonium chloride; ribosomal protein L35a
Dtna; ENSRNOG00000016671	Protein Dtna-ps1; dystrobrevin, alpha [Source:RGD Symbol;Acc:1561985]; dystrobrevin, alpha; ENCODES a protein that exhibits PDZ domain binding AND ASSOCIATED WITH

	Cardiomyopathies (ortholog) AND LEFT VENTRICULAR NONCOMPACTION 1 (ortholog) AND FOUND IN axon AND cell projection AND cytoplasm AND INTERACTS WITH cisplatin AND dibutyl phthalate AND 17beta-estradiol (ortholog); Uncharacterized protein [Source:UniProtKB/TrEMBL;Acc:D3ZD04]
RGD1563705	similar to ribosomal protein S23 [Source:RGD Symbol;Acc:1563705]; ENCODES a protein that exhibits structural constituent of ribosome (inferred) AND INVOLVED IN translation (inferred) AND FOUND IN small ribosomal subunit (inferred) AND INTERACTS WITH paracetamol (ortholog) AND quercetin (ortholog)
Glod5	glyoxalase domain containing 5
Nit2	nitrilase family, member 2
Penk	proenkephalin
Wt1	Wilms tumor 1
Trex1	three prime repair exonuclease 1
Col4a1	collagen, type IV, alpha 1
Asb4	ankyrin repeat and SOCS box-containing 4
Plekhg2	pleckstrin homology domain containing, family G (with RhoGef domain) member 2
Ppwd1	peptidylprolyl isomerase domain and WD repeat containing 1
LOC683573	INTERACTS WITH (-)-epigallocatechin 3-gallate (ortholog) AND 17beta-estradiol (ortholog) AND 2 3 7 8-tetrachlorodibenzodioxine (ortholog)
Fbln5	fibulin 5
Rd3l	retinal degeneration 3-like
Tdp2	tyrosyl-DNA phosphodiesterase 2
Rbck1	RanBP-type and C3HC4-type zinc finger containing 1
Capza2	capping protein (actin filament) muscle Z-line, alpha 2; capping protein (actin filament) muscle Z-line, alpha 2 (Capza2), mRNA; ENCODES a protein that exhibits actin binding (inferred) AND INVOLVED IN actin cytoskeleton organization (inferred) AND actin filament capping (inferred) AND FOUND IN cortical cytoskeleton (ortholog) AND extracellular vesicular exosome (ortholog) AND F-actin capping protein complex (ortholog) AND INTERACTS WITH

	2 6-dinitrotoluene AND 3H-1 2-dithiole-3-thione AND 7 12-dimethyltetraphene; capping protein (actin filament) muscle Z-line, alpha 2 (Capza2), mRNA,
Rpl19	ribosomal protein L19
Crabp2	cellular retinoic acid binding protein 2
Rhoh	ras homolog family member H
Chrna1	cholinergic receptor, nicotinic, alpha 1
Cript	cysteine-rich PDZ-binding protein
Olr1142; Olr1143	olfactory receptor 1142; olfactory receptor 1143
Cpt1a	carnitine palmitoyltransferase 1a, liver
Pdlim1	PDZ and LIM domain 1
Fam65b	family with sequence similarity 65, member B
Vsnl1	visinin-like 1
Tuba1b	tubulin, alpha 1B
Hyls1	hydrolethalus syndrome 1
Zfp37; LOC100363405; LOC102555249	zinc finger protein 37; Zfp37 pseudogene; ENCODES a protein that exhibits DNA binding (inferred) AND metal ion binding (inferred) AND nucleic acid binding (inferred) AND INVOLVED IN cell differentiation AND cell proliferation AND FOUND IN intracellular (inferred) AND nucleus (inferred) AND INTERACTS WITH 2 3 7 8-tetrachlorodibenzodioxine AND 3H-1 2-dithiole-3-thione AND ammonium chloride; zinc finger protein 37-like
Ecm1	extracellular matrix protein 1
Dnpep	aspartyl aminopeptidase; ENCODES a protein that exhibits aminopeptidase activity (inferred) AND metallopeptidase activity (inferred) AND zinc ion binding (inferred) AND INVOLVED IN proteolysis (inferred) AND FOUND IN blood microparticle (ortholog) AND nucleus (ortholog) AND INTERACTS WITH 2 3 7 8-tetrachlorodibenzodioxine AND N-methyl-N-nitrosourea AND (-)-epigallocatechin 3-gallate (ortholog); aspartyl aminopeptidase (Dnpep), mRNA,
Paqr4	progestin and adipoQ receptor family member IV
Kif11	kinesin family member 11
Nr2f2	nuclear receptor subfamily 2, group F, member 2
Acd	adrenocortical dysplasia homolog (mouse)
Dlx5	distal-less homeobox 5

Fignl1	fidgetin-like 1
Trim2	tripartite motif-containing 2
Hsdl2	hydroxysteroid dehydrogenase like 2
Fli1; LOC100910769	Fli-1 proto-oncogene, ETS transcription factor; Uncharacterized protein; ENCODES a protein that exhibits chromatin binding (ortholog) AND RNA polymerase II core promoter proximal region sequence-specific DNA binding (ortholog) AND RNA polymerase II core promoter proximal region sequence-specific DNA binding transcription factor activity involved in positive regulation of transcription (ortholog) AND INVOLVED IN blood circulation (ortholog) AND megakaryocyte development (ortholog) AND organ morphogenesis (ortholog) AND ASSOCIATED WITH Thrombocytopenia (ortholog) AND FOUND IN nucleus (ortholog) AND INTERACTS WITH 1 3-dinitrobenzene AND tributylstannane AND 17beta-hydroxy-17-methylestra-4 9 11-trien-3-one (ortholog); Friend leukemia integration 1 transcription factor-like
Car2	carbonic anhydrase 2
Reep4	receptor accessory protein 4
LOC688176	INTERACTS WITH copper(2+) sulfate (ortholog) AND hydrogen peroxide (ortholog) AND paracetamol (ortholog)
LOC100910418	tissue-type plasminogen activator-like
Opcml	opioid binding protein/cell adhesion molecule-like
Vom1r84; LOC100912389	vomeronasal 1 receptor 84; vomeronasal 1 receptor 84 (Vom1r84), mRNA; ENCODES a protein that exhibits pheromone receptor activity (inferred) AND INVOLVED IN G-protein coupled receptor signaling pathway (inferred) AND FOUND IN integral component of membrane (inferred)
Mns1	meiosis-specific nuclear structural 1
Cryl1	crystallin, lambda 1
LOC100910235	sulfotransferase 1C1-like [Source:RGD Symbol;Acc:6499500]; ENCODES a protein that exhibits sulfotransferase activity (inferred) AND INVOLVED IN metabolic process (inferred) AND INTERACTS WITH aflatoxin B1 (ortholog) AND benzo[a]pyrene (ortholog) AND bisphenol A (ortholog)
Alg3	ALG3, alpha-1,3- mannosyltransferase
Gatb	glutamyl-tRNA(Gln) amidotransferase, subunit B

Pf4	platelet factor 4
Ccnd3	cyclin D3
Apln	apelin
Hemgn	hemogen
Ptprc	protein tyrosine phosphatase, receptor type, C
Nap1l3	nucleosome assembly protein 1-like 3
LOC100362345	INTERACTS WITH calcitriol (ortholog) AND cyclosporin A (ortholog) AND ethyl methanesulfonate (ortholog)
Grn	granulin
Ephx1	epoxide hydrolase 1, microsomal (xenobiotic)
Tcf19	transcription factor 19
RGD1562987	similar to cDNA sequence BC031181
Chic1	Protein Chic1; cysteine-rich hydrophobic domain 1; INTERACTS WITH 3-methylcholanthrene (ortholog) AND benzo[a]pyrene (ortholog) AND calcitriol (ortholog)
Fcer2	Fc fragment of IgE, low affinity II, receptor for (CD23)
Hmcn2; LOC688582; LOC688599	Protein Hmcn2; hemicentin 2; similar to hemicentin 1; ENCODES a protein that exhibits calcium ion binding (inferred) AND FOUND IN basement membrane (ortholog) AND cell cortex (ortholog) AND cell junction (ortholog) AND INTERACTS WITH 17beta-estradiol (ortholog) AND benzo[a]pyrene (ortholog) AND choline (ortholog)
Dctn3	dynactin 3
Arntl	aryl hydrocarbon receptor nuclear translocator-like
Ccl5	chemokine (C-C motif) ligand 5
H1f0	H1 histone family, member 0
Hk1	hexokinase 1
Caskin2	cask-interacting protein 2
Skint8	selection and upkeep of intraepithelial T cells 8
Fgfr1op	Fgfr1 oncogene partner
Asb11	ankyrin repeat and SOCS box containing 11, E3 ubiquitin protein ligase
Lonrf3	LON peptidase N-terminal domain and ring finger 3
Pigp	phosphatidylinositol glycan anchor biosynthesis, class P
Nt5c3b	5'-nucleotidase, cytosolic IIIB
Itga2b	integrin, alpha 2B
Nmrk2	nicotinamide riboside kinase 2

E2f4	E2F transcription factor 4, p107/p130-binding
Pomp	proteasome maturation protein
Olr611	olfactory receptor 611
Mettl20	methyltransferase like 20
Gpr146	G protein-coupled receptor 146
Zdhhc4	zinc finger, DHHC-type containing 4
Cdc26	cell division cycle 26
Qsox1	quiescin Q6 sulfhydryl oxidase 1
Phf2	PHD finger protein 2
Rpl10l	ribosomal protein L10-like
LOC100912115; Ctage5	cutaneous T-cell lymphoma-associated antigen 5 homolog; CTAGE family, member 5; INTERACTS WITH 2 3 7 8-tetrachlorodibenzodioxine AND fipronil AND rotenone
Adamts10	ADAM metallopeptidase with thrombospondin type 1 motif, 10
RGD1566386	similar to Hypothetical protein A430033K04
Omd	osteomodulin
Prkcdbp	protein kinase C, delta binding protein
Rpl26	ribosomal protein L26
LOC100362296	protein S100-A11-like
Acsf2	acyl-CoA synthetase family member 2
Arl8b	ADP-ribosylation factor-like 8B
Slc29a2	solute carrier family 29 (equilibrative nucleoside transporter), member 2
F2rl2	coagulation factor II (thrombin) receptor-like 2
Emp1	epithelial membrane protein 1
Arl1	ADP-ribosylation factor-like 1
Racgap1	Rac GTPase-activating protein 1
Tgfb1i1	transforming growth factor beta 1 induced transcript 1
Anxa5	annexin A5
Klhl33; A930018M24Rik	kelch-like family member 33 [Source:RGD Symbol;Acc:2324282]; RIKEN cDNA A930018M24 gene [Source:MGI Symbol;Acc:MGI:2686053]; kelch-like family member 33
Cdipt	CDP-diacylglycerol--inositol 3-phosphatidyltransferase
Rpl36	ribosomal protein L36
Hmgn5	high mobility group nucleosome binding domain 5
LOC103690171;	melanoma-associated antigen 1-like; INTERACTS WITH 17beta-

RGD1561327	estradiol (ortholog) AND 2 3 7 8-tetrachlorodibenzodioxine (ortholog) AND 4-phenylbutyric acid (ortholog); similar to melanoma antigen family A, 10
Pfn1	profilin 1
Slc29a2; LOC100911721	solute carrier family 29 (equilibrative nucleoside transporter), member 2 (Slc29a2), mRNA; equilibrative nucleoside transporter 2-like
Tma7; LOC100911762	translational machinery associated 7 homolog (Tma7), mRNA; coiled-coil domain-containing protein 72-like
Llph	LLP homolog, long-term synaptic facilitation (Aplysia)
Cnot11	CCR4-NOT transcription complex, subunit 11
Ccdc127	coiled-coil domain containing 127
Atp1a2	ATPase, Na+/K+ transporting, alpha 2 polypeptide
Ccdc28b	coiled coil domain containing 28B
Cdc7	cell division cycle 7
Zfp770	zinc finger protein 770
Ift27	intraflagellar transport 27
LOC100361879; Atp5e	ATP synthase subunit epsilon, mitochondrial-like [Source:RGD Symbol;Acc:2319299]; ATP synthase, H+ transporting, mitochondrial F1 complex, epsilon subunit; ENCODES a protein that exhibits proton-transporting ATP synthase activity rotational mechanism (inferred) AND proton-transporting ATPase activity rotational mechanism (inferred) AND INVOLVED IN ATP synthesis coupled proton transport (inferred) AND FOUND IN mitochondrial proton-transporting ATP synthase complex catalytic core F(1) (inferred) AND INTERACTS WITH (S)-nicotine (ortholog) AND 17beta-estradiol (ortholog) AND acetylsalicylic acid (ortholog)
Pnpla8	patatin-like phospholipase domain containing 8
Golm1	golgi membrane protein 1 [Source:RGD Symbol;Acc:1589384]; golgi membrane protein 1; INVOLVED IN nucleus organization (ortholog) AND regulation of lipid metabolic process (ortholog) AND FOUND IN extracellular space (ortholog) AND extracellular vesicular exosome (ortholog) AND Golgi apparatus (ortholog) AND INTERACTS WITH 17alpha-ethynylestradiol AND 2-methoxyethanol AND dexamethasone

Srsf3	serine/arginine-rich splicing factor 3
Sgol2	shugoshin-like 2 (S, pombe)
Ldlr	low density lipoprotein receptor
Esd	esterase D
RGD1563581	similar to S100 calcium binding protein A11 (calizzarin) [Source:RGD Symbol;Acc:1563581]; ENCODES a protein that exhibits calcium ion binding (inferred) AND calcium-dependent protein binding (inferred) AND INVOLVED IN regulation of cell proliferation (inferred) AND INTERACTS WITH indole-3-methanol AND thioacetamide AND 17beta-estradiol (ortholog); similar to S100 calcium binding protein A11 (calizzarin)
RGD1561944	INTERACTS WITH cefaloridine
Lsmem1	leucine-rich single-pass membrane protein 1
Nr1d1	nuclear receptor subfamily 1, group D, member 1
Alas1	5-aminolevulinate synthase 1
Zfp472	zinc finger protein 472
Nqo2	NAD(P)H dehydrogenase, quinone 2
Col15a1	collagen, type XV, alpha 1
S100a11	S100 calcium binding protein A11
Ppm1k	protein phosphatase, Mg2+/Mn2+ dependent, 1K
Lrrc24	leucine rich repeat containing 24
Pcbp3	poly(rC) binding protein 3
Adcy2	adenylate cyclase 2 (brain)
Kcnj14	potassium channel, inwardly rectifying subfamily J, member 14
Olr136	olfactory receptor 136
Cspg4	chondroitin sulfate proteoglycan 4
Prc1	protein regulator of cytokinesis 1
Parpbp	PARP1 binding protein
Rhog	ras homolog family member G
Otub1	OTU deubiquitinase, ubiquitin aldehyde binding 1
Gpnmb	glycoprotein (transmembrane) nmb
Ly49si2	immunoreceptor Ly49si2; immunoreceptor Ly49si2 (Ly49si2), mRNA; immunoreceptor Ly49si2immunoreceptor Ly49si2; ENCODES a protein that exhibits carbohydrate binding (inferred)
Aspm	abnormal spindle microtubule assembly
F2rl1	coagulation factor II (thrombin) receptor-like 1

Ccdc186; ENSRNOG00000017018	coiled-coil domain containing 186; Uncharacterized protein [Source:UniProtKB/TrEMBL;Acc:D3Z9Y0]
Eif5a	eukaryotic translation initiation factor 5A
LOC102551311	afadin-like
Ist1	increased sodium tolerance 1 homolog (yeast)
RGD1305184	similar to CDNA sequence BC023105
Bche	butyrylcholinesterase
Inpp4b	inositol polyphosphate-4-phosphatase, type II
Atp6v0c	ATPase, H+ transporting, lysosomal V0 subunit C
Maoa	monoamine oxidase A
LOC691173	Uncharacterized protein; similar to cytoskeleton associated protein 2; INTERACTS WITH 2-methylcholine (ortholog) AND aflatoxin B1 (ortholog) AND bisphenol A (ortholog)
Ecsit	ECSIT signalling integrator
LOC363337	similar to RIKEN cDNA 1700081O22
Ccdc6	coiled-coil domain containing 6
LOC499240	similar to predicted gene ICRFP703B1614Q5,5; INTERACTS WITH amiodarone (ortholog)
Cwf19l2	CWF19-like 2, cell cycle control (S, pombe)
LOC100911238	proteasome maturation protein-like; INVOLVED IN proteasome assembly (ortholog) AND ASSOCIATED WITH Keratosis Linearis with Ichthyosis Congenita and Sclerosing Keratoderma (ortholog) AND FOUND IN cytoplasm (ortholog) AND nucleus (ortholog) AND INTERACTS WITH 17alpha-ethynylestradiol AND 2 3 7 8-tetrachlorodibenzodioxine AND 2 6-di-tert-butyl-4-methylphenol
Fam65a	family with sequence similarity 65, member A
Phykpl	5-phosphohydroxy-L-lysine phospho-lyase
LOC499235	LRRGT00141; ENCODES a protein that exhibits protein dimerization activity (ortholog) AND sequence-specific DNA binding (ortholog) AND sequence-specific DNA binding transcription factor activity (ortholog) AND INVOLVED IN glucose metabolic process (ortholog) AND negative regulation of apoptotic process (ortholog) AND negative regulation of cell proliferation (ortholog) AND FOUND IN nucleus (ortholog) AND INTERACTS WITH 17beta-estradiol (ortholog) AND 2 3 7 8-

	tetrachlorodibenzodioxine (ortholog) AND arsenite(3-) (ortholog); PREDICTED: LRRGT00141 (LOC499235), mRNA,
LOC100909481	eukaryotic translation initiation factor 3 subunit E-like
LOC100365062	eukaryotic translation initiation factor 3 subunit 6 48kDa-like
Srm	ENCODES a protein that exhibits spermidine synthase activity AND protein homodimerization activity (ortholog) AND INVOLVED IN spermidine biosynthetic process AND PARTICIPATES IN spermidine metabolic pathway AND methionine cycle/metabolic pathway AND polyamine metabolic pathway AND INTERACTS WITH 2 3 7 8-tetrachlorodibenzodioxine AND 2 4 6-trinitrotoluene AND 2 4-dinitrotoluene; spermidine synthase; spermidine synthase (Srm), mRNA,
Arhgap1	Rho GTPase activating protein 1
Sipa1	signal-induced proliferation-associated 1
Ccdc18	coiled-coil domain containing 18 [Source:RGD Symbol;Acc:1564165]; Protein Ccdc18; coiled-coil domain containing 18; INTERACTS WITH (-)-epigallocatechin 3-gallate (ortholog) AND 1-nitropyrene (ortholog) AND 2 3 7 8-tetrachlorodibenzodioxine (ortholog); Uncharacterized protein [Source:UniProtKB/TrEMBL;Acc:D3ZKA3]
LOC100362479	ribosomal protein L15-like
Pfn2	profilin 2
Fetub	fetuin B
Anpep	alanyl (membrane) aminopeptidase
Ckb	creatine kinase, brain
Diaph3	diaphanous-related formin 3 [Source:RGD Symbol;Acc:1593287]; diaphanous-related formin 3; ENCODES a protein that exhibits actin binding (inferred) AND Rho GTPase binding (inferred) AND INVOLVED IN spermatogenesis AND ASSOCIATED WITH Auditory Neuropathy Autosomal Dominant 1 (ortholog) AND FOUND IN cytoplasm AND nucleus AND INTERACTS WITH cisplatin AND indole-3-methanol AND N-nitrosodiethylamine; PREDICTED: diaphanous homolog 3 (Drosophila), transcript variant 1 (Diaph3), mRNA,; PREDICTED: diaphanous homolog 3 (Drosophila), transcript variant 2 (Diaph3), mRNA,

RT1-Bb	RT1 class II, locus Bb
Slc52a3	solute carrier family 52, riboflavin transporter, member 3
Rtn1	reticulon 1
Vash2	vasohibin 2
LOC100909409; LOC688649	disks large homolog 5-like [Source:RGD Symbol;Acc:6504293]; similar to spermatogenesis associated glutamate (E)-rich protein 4d
Shisa5	shisa family member 5
Mall	mal, T-cell differentiation protein-like
Vat1	vesicle amine transport 1
Dad1	defender against cell death 1
Pc	pyruvate carboxylase
Rad54b	RAD54 homolog B (S, cerevisiae)
Tnp1	transition protein 1
Nid2	nidogen 2 (osteonidogen)
Camk2d	calcium/calmodulin-dependent protein kinase II delta
Kcnj2	potassium channel, inwardly rectifying subfamily J, member 2
Vgll4	vestigial-like family member 4
Emb	embigin
Lrrc39	leucine rich repeat containing 39
Per1	period circadian clock 1
Dnm3	dynamin 3
Dmd	dystrophin
Ciart	circadian associated repressor of transcription; ENCODES a protein that exhibits core promoter sequence-specific DNA binding (ortholog) AND E-box binding (ortholog) AND INVOLVED IN circadian regulation of gene expression (ortholog) AND locomotor rhythm (ortholog) AND negative regulation of transcription DNA-templated (ortholog) AND FOUND IN nucleus (ortholog) AND INTERACTS WITH (-)-epigallocatechin 3-gallate (ortholog) AND arsenite(3-) (ortholog) AND cadmium atom (ortholog)
LOC688684	similar to 60S ribosomal protein L32 [Source:RGD Symbol;Acc:1586416]; similar to 60S ribosomal protein L32; ENCODES a protein that exhibits structural constituent of ribosome (inferred) AND INVOLVED IN translation (inferred) AND INTERACTS WITH 17beta-estradiol (ortholog) AND copper(II)

	chloride (ortholog) AND disodium selenite (ortholog)
Tmem204	transmembrane protein 204
Ccr2	chemokine (C-C motif) receptor 2
Tp53	tumor protein p53
RGD1565131	similar to ribosomal protein L15
Pprc1	peroxisome proliferator-activated receptor gamma, coactivator-related 1
Aqp7	aquaporin 7
Bcl2l11	BCL2-like 11 (apoptosis facilitator)
Tmem47	transmembrane protein 47
Arhgap11a	Rho GTPase activating protein 11A
Cyp2e1	cytochrome P450, family 2, subfamily e, polypeptide 1
Ccnb2	cyclin B2; ENCODES a protein that exhibits protein kinase binding (inferred) AND INVOLVED IN growth (ortholog) AND in utero embryonic development (ortholog) AND T cell homeostasis (ortholog) AND PARTICIPATES IN cell cycle pathway mitotic AND p53 signaling pathway AND FOUND IN centrosome (ortholog) AND membrane (ortholog) AND microtubule cytoskeleton (ortholog) AND INTERACTS WITH 17alpha-ethynylestradiol AND 2-acetamidofluorene AND cefaloridine
Mmrn1	multimerin 1
Rgcc	regulator of cell cycle
Abra	actin-binding Rho activating protein
Cd276	Cd276 molecule
Il2rg	interleukin 2 receptor, gamma
LOC681193	ENCODES a protein that exhibits DNA binding (inferred) AND DNA-directed RNA polymerase activity (inferred) AND zinc ion binding (inferred) AND INVOLVED IN transcription DNA-templated (inferred) AND INTERACTS WITH 5-aza-2'-deoxycytidine (ortholog) AND cisplatin (ortholog) AND cobalt dichloride (ortholog)
Stxbp1	syntaxin binding protein 1
Smox	spermine oxidase
Ifi27	interferon, alpha-inducible protein 27
Cxcl12	chemokine (C-X-C motif) ligand 12
Mum1l1	melanoma associated antigen (mutated) 1-like 1

Olr59	olfactory receptor 59
Tpm4	tropomyosin 4
Spdya	speedy/RINGO cell cycle regulator family member A
Prdx3	peroxiredoxin 3
Chi3l3	chitinase 3-like 3
Entpd2	ectonucleoside triphosphate diphosphohydrolase 2
Ddx11	DEAD/H (Asp-Glu-Ala-Asp/His) box helicase 11
Wdr83os	WD repeat domain 83 opposite strand
Naaladl2	N-acetylated alpha-linked acidic dipeptidase-like 2
Rab2b	RAB2B, member RAS oncogene family
Rin3	Ras and Rab interactor 3
Nusap1	nucleolar and spindle associated protein 1
Chrac1	chromatin accessibility complex 1
Txn1	thioredoxin 1
Hadha	hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/enoyl-CoA hydratase (trifunctional protein), alpha subunit
Ednrb	endothelin receptor type B
RGD1560860; LOC100912220	similar to ankyrin repeat domain 26; uncharacterized LOC100912220
Tmem177	transmembrane protein 177
Stim2	stromal interaction molecule 2
Ssh2	slingshot protein phosphatase 2
Zfp667	zinc finger protein 667
Myadm	myeloid-associated differentiation marker
Olr1301	ENCODES a protein that exhibits olfactory receptor activity (inferred) AND INVOLVED IN detection of chemical stimulus involved in sensory perception of smell (inferred) AND G-protein coupled receptor signaling pathway (inferred) AND FOUND IN integral component of membrane (inferred)
Plxdc1	plexin domain containing 1
Nfu1	NFU1 iron-sulfur cluster scaffold
Exnef	exonuclease NEF-sp
RGD1311343	similar to RIKEN cDNA 4930524B15
Rpp14	ribonuclease P/MRP 14 subunit
Ptges3	prostaglandin E synthase 3 (cytosolic)
Spata9	spermatogenesis associated 9

Wsb2	WD repeat and SOCS box-containing 2
Sumo1	small ubiquitin-like modifier 1
Mki67	marker of proliferation Ki-67
Rbp7	retinol binding protein 7, cellular
Pfkfb1	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 1
Atp6ap1	ATPase, H+ transporting, lysosomal accessory protein 1
Tecta	tectorin alpha
NONMMUG024767	Non-coding transcript identified by NONCODE: Exonic
Arrdc1	arrestin domain containing 1
Tubb4b	tubulin, beta 4B class IVb
Gse1	Gse1 coiled-coil protein [Source:RGD Symbol;Acc:1562686]; Protein Gse1; Gse1 coiled-coil protein; INTERACTS WITH 3H-1 2-dithiole-3-thione AND all-trans-retinoic acid AND dibutyl phthalate
Col15a1	collagen, type XV, alpha 1 [Source:RGD Symbol;Acc:1310820]; ENCODES a protein that exhibits structural molecule activity (inferred) AND INVOLVED IN cell adhesion (inferred) AND PARTICIPATES IN syndecan signaling pathway AND FOUND IN basement membrane (ortholog) AND extracellular matrix (ortholog) AND extracellular space (ortholog) AND INTERACTS WITH 2 3 7 8-tetrachlorodibenzodioxine AND cocaine AND dexamethasone
Srm	spermidine synthase
Plat	plasminogen activator, tissue
Hcfc1r1	host cell factor C1 regulator 1 (XPO1-dependent)
Col1a2	collagen, type I, alpha 2
Ifngr2	interferon gamma receptor 2
LOC102554136	zinc finger protein 60-like
Eif3m	eukaryotic translation initiation factor 3, subunit M
Syt5	synaptotagmin V
Cks2	CDC28 protein kinase regulatory subunit 2
Calcoco1	calcium binding and coiled coil domain 1
Atp5e	ATP synthase, H+ transporting, mitochondrial F1 complex, epsilon subunit
Ifna16l1	INTERACTS WITH 1-nitropyrene (ortholog) AND 2 3 7 8-tetrachlorodibenzodioxine (ortholog) AND 5-aza-2'-deoxycytidine (ortholog)

Sypl2	synaptophysin-like 2
LOC100910079	actin-related protein 3B-like
Vom1r68; Vom1r67; Vom1r66; Vom1r71; Vom1r69	vomeronasal 1 receptor 68; vomeronasal 1 receptor 67; vomeronasal 1 receptor 66; vomeronasal 1 receptor 71; vomeronasal 1 receptor 69
Omd; LOC102550506	osteomodulin (Omd), mRNA; INTERACTS WITH 2 3 7 8-tetrachlorodibenzodioxine AND 3H-1 2-dithiole-3-thione AND ammonium chloride; osteomodulin
MGC94199; LOC100910681	similar to RIKEN cDNA 2610301B20; EST AI428449 (MGC94199), mRNA; similar to RIKEN cDNA 2610301B20; EST AI428449 [Source:RGD Symbol;Acc:1549749]; similar to RIKEN cDNA 2610301B20; EST AI428449, mRNA (cDNA clone MGC:94199 IMAGE:7129828), complete cds,; similar to RIKEN cDNA 2610301B20; EST AI428449; INVOLVED IN visual perception (inferred) AND FOUND IN cell junction (ortholog) AND cytoplasm (ortholog) AND plasma membrane (ortholog); protein C8orf37 homolog; similar to RIKEN cDNA 2610301B20; EST AI428449 (MGC94199), mRNA,
Dexi	dexamethasone-induced transcript
Pfn2; LOC100909840	profilin 2 (Pfn2), mRNA; ENCODES a protein that exhibits actin binding (inferred) AND INVOLVED IN actin cytoskeleton organization (inferred) AND INTERACTS WITH 3 3' 4 4' 5-pentachlorobiphenyl (ortholog) AND 5-fluorouracil (ortholog) AND cyclosporin A (ortholog)
Plscr3	phospholipid scramblase 3
Fgd6	FYVE, RhoGEF and PH domain containing 6
Rarres1	retinoic acid receptor responder (tazarotene induced) 1
Myof	myoferlin
Il1b	interleukin 1 beta
Nudt7	nudix (nucleoside diphosphate linked moiety X)-type motif 7
Klf5	Kruppel-like factor 5
Ctsb	cathepsin B
Fto	fat mass and obesity associated
Praf2	PRA1 domain family, member 2
Zwilch	zwilch kinetochore protein
Plk1	polo-like kinase 1

Capns1	calpain, small subunit 1
Ramp2	receptor (G protein-coupled) activity modifying protein 2
RGD1561161	similar to BC067074 protein
Zfp563	zinc finger protein 563
Psmf1	proteasome (prosome, macropain) inhibitor subunit 1
Slc39a11	solute carrier family 39, member 11
LOC690131; Hist2h2aa2	similar to H2A histone family, member O; ENCODES a protein that exhibits DNA binding (inferred) AND protein heterodimerization activity (inferred) AND INVOLVED IN nucleosome assembly (inferred) AND FOUND IN extracellular vesicular exosome (ortholog) AND nucleus (ortholog) AND INTERACTS WITH 2 3 7 8-tetrachlorodibenzodioxine AND 7 12-dimethyltetraphene AND cisplatin
Clec4a	C-type lectin domain family 4, member A
RT1-Db1	RT1 class II, locus Db1
Klrb1c	killer cell lectin-like receptor subfamily B member 1C
Fads2	fatty acid desaturase 2
Eif1a	eukaryotic translation initiation factor 1A
Cited4	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 4
Commd2	COMM domain containing 2
Fbn1	fibrillin 1
Ntn4	netrin 4
Naa25	N(alpha)-acetyltransferase 25, NatB auxiliary subunit
Bex4	brain expressed, X-linked 4
Ptgfr	prostaglandin F receptor
F2r	coagulation factor II (thrombin) receptor
Mif	macrophage migration inhibitory factor (glycosylation-inhibiting factor)
Ctsa	cathepsin A
Rbm4	Chalmel, et, al, AceView Annotation Rbm4,gSep08
Dennd3	DENN/MADD domain containing 3
Vac14	Vac14 homolog (S, cerevisiae)
Nt5c1a	5-nucleotidase, cytosolic IA
Eif6	eukaryotic translation initiation factor 6
Rock2	Rho-associated coiled-coil containing protein kinase 2

Tmem255b	transmembrane protein 255B
Bex1	brain expressed, X-linked 1
Mrps10	mitochondrial ribosomal protein S10
Mif	macrophage migration inhibitory factor (glycosylation-inhibiting factor) [Source:RGD Symbol;Acc:621163]; macrophage migration inhibitory factor (glycosylation-inhibiting factor); ENCODES a protein that exhibits protein complex binding AND chemoattractant activity (ortholog) AND cytokine activity (ortholog) AND INVOLVED IN aging AND brain development AND brain renin-angiotensin system AND PARTICIPATES IN phenylalanine metabolic pathway AND tyrosine metabolic pathway AND ASSOCIATED WITH Anti-Glomerular Basement Membrane Disease AND Arthritis Experimental AND Asthma AND FOUND IN cytoplasm AND extracellular space AND nucleus AND INTERACTS WITH 1 2 4-trimethylbenzene AND 17alpha-ethynylestradiol AND 2 4 6-trinitrotoluene; macrophage migration inhibitory factor (Mif), mRNA,
Ddah2	dimethylarginine dimethylaminohydrolase 2
Vps29	VPS29 retromer complex component
Ryr3	Protein Ryr3; ryanodine receptor 3 [Source:RGD Symbol;Acc:68952]; ENCODES a protein that exhibits calcium-induced calcium release activity AND ryanodine-sensitive calcium-release channel activity AND calcium-release channel activity (ortholog) AND INVOLVED IN calcium ion transmembrane transport AND negative regulation of cytosolic calcium ion concentration AND calcium ion transport (ortholog) AND PARTICIPATES IN calcium transport pathway AND calcium/calcium-mediated signaling pathway AND Alzheimer disease pathway AND FOUND IN endoplasmic reticulum AND perinuclear region of cytoplasm AND junctional membrane complex (ortholog) AND INTERACTS WITH C60 fullerene AND cocaine AND tetrachloromethane; ryanodine receptor 3; Uncharacterized protein [Source:UniProtKB/TrEMBL;Acc:F1LPJ2]
Srp68	signal recognition particle 68
Ece2	endothelin-converting enzyme 2
Snap91	synaptosomal-associated protein 91

LOC100911363	ENCODES a protein that exhibits calcium ion binding (inferred) AND INTERACTS WITH 2 3 7 8-tetrachlorodibenzodioxine (ortholog) AND 3 3' 5-triiodo-L-thyronine (ortholog) AND 4-hydroxynon-2-enal (ortholog)
Rps6kb2	ribosomal protein S6 kinase, polypeptide 2
Slc35c2	solute carrier family 35 (GDP-fucose transporter), member C2
Cd302	CD302 molecule
Tbc1d25	TBC1 domain family, member 25
Tet1	tet methylcytosine dioxygenase 1
Klf10	Kruppel-like factor 10
Ang; Rnase4	angiogenin, ribonuclease, RNase A family, 5; ribonuclease, RNase A family 4
Mt1a	metallothionein 1a
Kcnk6	potassium channel, subfamily K, member 6 (Kcnk6), mRNA; potassium channel, two pore domain subfamily K, member 6 [Source:RGD Symbol;Acc:621450]; ENCODES a protein that exhibits potassium channel activity AND INVOLVED IN potassium ion transmembrane transport (inferred) AND FOUND IN integral component of membrane (inferred) AND INTERACTS WITH 17beta-estradiol (ortholog) AND diarsenic trioxide (ortholog) AND lithium chloride (ortholog)
Rpl15	ribosomal protein L15
Cish	cytokine inducible SH2-containing protein
Top2a	topoisomerase (DNA) II alpha
Dpp3	dipeptidylpeptidase 3
Mpi	mannose phosphate isomerase (mapped); ENCODES a protein that exhibits mannose-6-phosphate isomerase activity (ortholog) AND INVOLVED IN carbohydrate metabolic process (inferred) AND GDP-mannose biosynthetic process (inferred) AND PARTICIPATES IN amino sugar metabolic pathway AND fructose and mannose metabolic pathway AND nucleotide sugar metabolic pathway AND ASSOCIATED WITH Carbohydrate Metabolism Inborn Errors (ortholog) AND Congenital disorder of glycosylation type 1B (ortholog) AND FOUND IN extracellular vesicular exosome (ortholog) AND INTERACTS WITH (-)-epigallocatechin 3-gallate (ortholog) AND 3-methylcholanthrene (ortholog) AND

	amiodarone (ortholog); mannose phosphate isomerase (mapped) (Mpi), mRNA,
Lxn	latexin
Kif18a	kinesin family member 18A
Mad2l2	MAD2 mitotic arrest deficient-like 2 (yeast)
Tagln2	transgelin 2
Lactb2	lactamase, beta 2
Fgb	fibrinogen beta chain
Cdca2	cell division cycle associated 2
Cct4	chaperonin containing Tcp1, subunit 4 (delta)
LOC102546324	RBPJ-interacting and tubulin-associated protein-like
Gpr4	G protein-coupled receptor 4
Cdc37	cell division cycle 37
Tob1	transducer of ErbB-2,1
Itih6	Protein LOC100912775
Wdr89	WD repeat domain 89
Raet1c	Protein LOC679825; INVOLVED IN antigen processing and presentation (inferred) AND immune response (inferred) AND FOUND IN membrane (inferred)
LOC501396	INTERACTS WITH bilirubin (ortholog) AND bisphenol A (ortholog) AND copper atom (ortholog)
Ppp1r3d	protein phosphatase 1, regulatory subunit 3D
Nrarp	Notch-regulated ankyrin repeat protein
Lrrc8d	leucine rich repeat containing 8 family, member D
Chrna6	cholinergic receptor, nicotinic, alpha 6 (neuronal)
LOC679149	similar to carboxylesterase 2 (intestine, liver); similar to carboxylesterase 2 (intestine, liver) [Source:RGD Symbol;Acc:1591847]; null
Eva1c	eva-1 homolog C
Entpd1	ENCODES a protein that exhibits nucleoside-diphosphatase activity (ortholog) AND nucleoside-triphosphatase activity (ortholog) AND INVOLVED IN ATP catabolic process (ortholog) AND G-protein coupled receptor signaling pathway (ortholog) AND platelet activation (ortholog) AND PARTICIPATES IN purine metabolic pathway AND pyrimidine metabolic pathway AND ASSOCIATED WITH Epilepsy Partial Sensory (ortholog) AND

	Hemorrhage (ortholog) AND FOUND IN basal lamina (ortholog) AND extracellular vesicular exosome (ortholog) AND membrane (ortholog) AND INTERACTS WITH 2 4-dinitrotoluene AND ammonium chloride AND dibenzofuran
Klf8	Kruppel-like factor 8
RGD1562776	INTERACTS WITH C60 fullerene
Cstf3	cleavage stimulation factor, 3 pre-RNA, subunit 3, 77kDa
LOC502908	Protein LOC502908; INTERACTS WITH arsenic atom (ortholog)
Tpm3	tropomyosin 3
Fbxo44	F-box protein 44
Trim5	tripartite motif-containing 5
Rab5c	RAB5C, member RAS oncogene family
Mmachc	methylmalonic aciduria (cobalamin deficiency) cblC type, with homocystinuria
Armc7	armadillo repeat containing 7
Sh2d1b	SH2 domain containing 1B
Rab35	RAB35, member RAS oncogene family
Thegl	theg spermatid protein-like
Ifitm3	interferon induced transmembrane protein 3; INVOLVED IN cardiac muscle cell differentiation AND heart development AND defense response to virus (ortholog) AND FOUND IN apical part of cell (ortholog) AND cell surface (ortholog) AND cytoplasm (ortholog) AND INTERACTS WITH 17alpha-ethynylestradiol AND 2 3 7 8-tetrachlorodibenzodioxine AND 3 4-dichloroaniline; interferon induced transmembrane protein 3 (Ifitm3), mRNA,
Chpt1	choline phosphotransferase 1
Sec31a	SEC31 homolog A, COPII coat complex component
Prtfdc1	phosphoribosyl transferase domain containing 1
Alg13	ALG13, UDP-N-acetylglucosaminyltransferase subunit
Tk1	thymidine kinase 1, soluble
RT1-Ba	RT1 class II, locus Ba
Gpat3	glycerol-3-phosphate acyltransferase 3
Med15	mediator complex subunit 15
Babam1	BRISC and BRCA1 A complex member 1
Junb	jun B proto-oncogene
P3h2	prolyl 3-hydroxylase 2

Cnpy1	canopy FGF signaling regulator 1 [Source:RGD Symbol;Acc:1583296]; canopy FGF signaling regulator 1; INTERACTS WITH valproic acid (ortholog)
Ndufa4l2	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 4-like 2
Rmdn1	regulator of microtubule dynamics 1
Arl2	ADP-ribosylation factor-like 2
Wasf2	WAS protein family, member 2
Glrx	glutaredoxin (thioltransferase)
Zfp113	zinc finger protein 3
Csdc2	cold shock domain containing C2, RNA binding
Osbp2	oxysterol binding protein 2
Ankrd45	ankyrin repeat domain 45 [Source:RGD Symbol;Acc:1311153]; ankyrin repeat domain 45; PREDICTED: ankyrin repeat domain 45 (Ankrd45), mRNA,
Ggt5	ENCODES a protein that exhibits gamma-glutamyltransferase activity (ortholog) AND INVOLVED IN response to organic nitrogen AND inflammatory response (ortholog) AND leukotriene biosynthetic process (ortholog) AND PARTICIPATES IN arachidonic acid metabolic pathway AND cyanoamino acid metabolic pathway AND glutathione metabolic pathway AND ASSOCIATED WITH Lung Neoplasms AND FOUND IN plasma membrane (ortholog) AND INTERACTS WITH ochratoxin A AND pirinixic acid AND 2 3 7 8-tetrachlorodibenzodioxine (ortholog)
Capg	capping protein (actin filament), gelsolin-like
Klrc1	killer cell lectin-like receptor subfamily C, member 1
Alas2	5-aminolevulinate synthase 2
Atp1a3	ATPase, Na+/K+ transporting, alpha 3 polypeptide
Bola1	bolA family member 1
Olr341	olfactory receptor 341
Tpx2	TPX2, microtubule-associated
Entpd1	ectonucleoside triphosphate diphosphohydrolase 1
Ccna2	cyclin A2
ENSMUSG00000045750; ENSMUSG00000046079; NONMMUG033211	RIKEN cDNA D830014E11 gene, leucine rich repeat containing 8D; leucine rich repeat containing 8D; Non-coding transcript identified by NONCODE: Exonic

Mamdc2	MAM domain containing 2
Acad10	acyl-CoA dehydrogenase family, member 10
Kif4a	kinesin family member 4A
Txlng	taxilin gamma
Apmap	adipocyte plasma membrane associated protein
Cxcl11	chemokine (C-X-C motif) ligand 11
Lpar6	lysophosphatidic acid receptor 6
Cxadr	coxsackie virus and adenovirus receptor
Mt1a	metallothionein 1a
Mboat2	membrane bound O-acyltransferase domain containing 2
Tfrc	transferrin receptor
Akr1cl	aldo-keto reductase family 1, member C-like
Pcdh11x; LOC100910387	protocadherin 11 X-linked; protocadherin 11 X-linked [Source:RGD Symbol;Acc:1562864]; protocadherin-11 X-linked-like; ENCODES a protein that exhibits calcium ion binding (inferred) AND INVOLVED IN negative regulation of phosphatase activity (ortholog) AND FOUND IN extracellular vesicular exosome (ortholog) AND INTERACTS WITH 17beta-estradiol (ortholog) AND aflatoxin B1 (ortholog) AND all-trans-retinoic acid (ortholog)
RT1-S3	RT1 class Ib, locus S3
Kitlg	KIT ligand
LOC499718; LOC102555814	hypothetical LOC499718; uncharacterized LOC102555814
P2ry1	purinergic receptor P2Y, G-protein coupled, 1
Tacc3	transforming, acidic coiled-coil containing protein 3
Ifitm1	interferon induced transmembrane protein 1
Cdc42ep2	CDC42 effector protein (Rho GTPase binding) 2
Coq3	coenzyme Q3 methyltransferase
RGD1560017	ENCODES a protein that exhibits structural constituent of ribosome (inferred) AND INVOLVED IN translation (inferred) AND FOUND IN large ribosomal subunit (inferred)
Itga9	integrin, alpha 9 [Source:RGD Symbol;Acc:1311191]; integrin, alpha 9; ENCODES a protein that exhibits collagen binding AND laminin binding AND INVOLVED IN wound healing AND cell adhesion (ortholog) AND neutrophil chemotaxis (ortholog) AND PARTICIPATES IN altered integrin mediated signaling pathway

	AND integrin mediated signaling pathway AND pancreatic cancer pathway AND ASSOCIATED WITH Pancreatic Neoplasms (ortholog) AND FOUND IN integrin alpha9-beta1 complex AND membrane AND basal plasma membrane (ortholog) AND INTERACTS WITH 2 3 7 8-tetrachlorodibenzodioxine AND cisplatin AND phenylephrine
Litaf	lipopolysaccharide-induced TNF factor
Lamc1	laminin, gamma 1
Gbp1	guanylate binding protein 1, interferon-inducible [Source:RGD Symbol;Acc:1311877]
Ubl4a	ubiquitin-like 4A
Midn	midnolin
Racgap1	Rac GTPase-activating protein 1
Cds1	CDP-diacylglycerol synthase 1
Ecm2	ENCODES a protein that exhibits collagen binding (ortholog) AND collagen V binding (ortholog) AND heparin binding (ortholog) AND INVOLVED IN extracellular matrix organization (ortholog) AND positive regulation of cell-substrate adhesion (ortholog) AND FOUND IN extracellular matrix (ortholog) AND interstitial matrix (ortholog) AND INTERACTS WITH 17alpha-ethynylestradiol AND diuron AND dopamine
Tgfb1	transforming growth factor, beta 1
RGD1566137; LOC100910950	Ribosomal protein; ENCODES a protein that exhibits RNA binding (inferred) AND structural constituent of ribosome (inferred) AND INVOLVED IN translation (inferred) AND FOUND IN large ribosomal subunit (inferred) AND INTERACTS WITH 5-fluorouracil (ortholog) AND copper(2+) sulfate (ortholog) AND diarsenic trioxide (ortholog); 60S ribosomal protein L10a-like
Mst1r	macrophage stimulating 1 receptor
Ypel3	yippee-like 3
Acox1	acyl-CoA oxidase 1, palmitoyl
S1pr1	sphingosine-1-phosphate receptor 1
LOC100909497	putative PRAME family member 24-like
Plk2	polo-like kinase 2
Tecrl	trans-2,3-enoyl-CoA reductase-like
Ufc1	ubiquitin-fold modifier conjugating enzyme 1

Adamts12	ADAM metallopeptidase with thrombospondin type 1 motif, 12
Pnp	purine nucleoside phosphorylase
Nebl	nebulette
Ptpn12	protein tyrosine phosphatase, non-receptor type 12
LOC100911204	protein CASC5-like; INTERACTS WITH 17beta-hydroxy-17-methylestra-4 9 11-trien-3-one (ortholog) AND 3 4-dichloroaniline (ortholog) AND calcitriol (ortholog)
Cacng6	calcium channel, voltage-dependent, gamma subunit 6
H2afx	H2A histone family, member X
LOC100361139; Thymosin,0	ENCODES a protein that exhibits actin binding (inferred) AND INVOLVED IN actin cytoskeleton organization (inferred) AND sequestering of actin monomers (inferred) AND FOUND IN cytoplasm (inferred); Chalmel, et, al, AceView Annotation Thymosin,0,aSep08
LOC683674; LOC102555075; Zfp853	similar to Protein C7orf26 homolog [Source:RGD Symbol;Acc:1584479]; INTERACTS WITH (S)-colchicine (ortholog) AND adenine (ortholog) AND cisplatin (ortholog); uncharacterized LOC102555075; zinc finger protein 853
Reg3g	regenerating islet-derived 3 gamma
Kcnj5	potassium channel, inwardly rectifying subfamily J, member 5
Cdc42ep1	CDC42 effector protein (Rho GTPase binding) 1
Cdk1	cyclin-dependent kinase 1
Synm	synemin, intermediate filament protein
Unc119b	unc-119 lipid binding chaperone B
Pcdh12	protocadherin 12
Zfp955a	zinc finger protein 955A; ENCODES a protein that exhibits metal ion binding (inferred) AND nucleic acid binding (inferred) AND INVOLVED IN regulation of transcription DNA-templated (inferred) AND FOUND IN intracellular (inferred) AND INTERACTS WITH arsenite(3-) (ortholog)
Gria4	glutamate receptor, ionotropic, AMPA 4
Zfp36l1	zinc finger protein 36, C3H type-like 1
Olr898	olfactory receptor 898
Hspb1	heat shock protein B1
H2afj	H2A histone family, member J
LOC100361098	28S ribosomal protein L42, mitochondrial-like; INTERACTS WITH

	2-methylcholine (ortholog) AND all-trans-retinoic acid (ortholog) AND cobalt dichloride (ortholog)
Aif1l	allograft inflammatory factor 1-like
Stxbp6	syntaxin binding protein 6 (amisyn)
Dcakd	dephospho-CoA kinase domain containing
Adipoq	adiponectin, C1Q and collagen domain containing
Snca	synuclein, alpha (non A4 component of amyloid precursor)
Mtg2	mitochondrial ribosome-associated GTPase 2
Cetn3	centrin, EF-hand protein, 3 (Cetn3), mRNA; centrin, EF-hand protein, 3 [Source:RGD Symbol;Acc:620249]; ENCODES a protein that exhibits G-protein beta/gamma-subunit complex binding (ortholog) AND microtubule binding (ortholog) AND FOUND IN centriole (ortholog) AND centrosome (ortholog) AND ciliary basal body (ortholog) AND INTERACTS WITH 2 4-dinitrotoluene AND 2 6-dinitrotoluene AND ammonium chloride
Gria3	glutamate receptor, ionotropic, AMPA 3
Hspa12b	heat shock protein 12B
St6gal1	ST6 beta-galactosamide alpha-2,6-sialyltranferase 1
Fam214b	family with sequence similarity 214, member B
Depdc1	DEP domain containing 1
MGC94199; LOC102554232	similar to RIKEN cDNA 2610301B20; EST AI428449; similar to RIKEN cDNA 2610301B20; EST AI428449 [Source:RGD Symbol;Acc:1549749]; protein C8orf37 homolog
Tmem246	transmembrane protein 246
Srp68	signal recognition particle 68
Itm2a	integral membrane protein 2A
F13a1	coagulation factor XIII, A1 polypeptide
Pgam1	phosphoglycerate mutase 1 (brain)
E4f1	E4F transcription factor 1
Vom1r40	vomeronasal 1 receptor 40
Vom1r40; LOC102551504	vomeronasal 1 receptor 40 (Vom1r40), mRNA; vomeronasal type-1 receptor 4-like; vomeronasal 1 receptor 40
Clstn2	calsyntenin 2; calsyntenin 2 (Clstn2), mRNA, NM_134377; ENCODES a protein that exhibits calcium ion binding (inferred) AND INVOLVED IN homophilic cell adhesion (inferred) AND FOUND IN postsynaptic membrane AND INTERACTS WITH

	17beta-estradiol AND benzo[a]pyrene AND cefaloridine
Zmat1	zinc finger, matrin-type 1
Nfil3	nuclear factor, interleukin 3 regulated
Sf3a2	splicing factor 3a, subunit 2
Tomm40	translocase of outer mitochondrial membrane 40 homolog (yeast)
RGD1561778	similar to dendritic cell-derived immunoglobulin(Ig)-like receptor 1, DIgR1 - mouse
Myct1	myc target 1
Plp1	proteolipid protein 1
Mospd3	motile sperm domain containing 3
Rhoj	ras homolog family member J
Hdac5	histone deacetylase 5
Phf24	PHD finger protein 24

ARTIGO 2

Translation and cultural adaptation of the SYRCLE risk of bias tool for Brazilian Portuguese

Francis Lopes Pacagnelli[1], Thaoan Bruno Mariano[1], Ines Cristina Giometti, Robson Chacon Castoldi, Ana Paula Coelho Figueira Freire[1,2], Larissa Ferreira Rós Mariano, Glaura Scantamburlo Alves Fernandes[3], Andréia Gomes Bezerra[4], Gabriel Natan Pires[4,5].

1- Department of Physiotherapy, Universidade do Oeste Paulista, Presidente Prudente, SP, Brazil

2- Central Washington University, Washington, USA.

3- Department of Pathology, State University of Londrina, Londrina, PR, Brazil

6- Department of Psychobiology, Universidade Federal de São Paulo, São Paulo, Brazil.

7- Brazilian Reproducibility Initiative in preclinical Systematic review and meta-Analysis – Rio de Janeiro, Brazil.

Abstract

Introduction: Experimental research using animal models is commonly conducted in Brazil and around the world; findings from these studies are often included in systematic reviews. One of the fundamental processes during the addition of studies to systematic reviews is the analysis of risk of bias. SYRCLE (Systematic Review Center for Laboratory Animal Experimentation, Nijmegen, Netherlands), a major international research group promoting the systematic reviews of studies, is responsible for the main tool for assessing the risk of methodological bias in animal model studies (SYRCLE RoB). However, this tool is only available in English. Its translation and cultural validation in Portuguese would disseminate and encourage researchers to conduct systematic reviews in experimental animal models. Objective: To translate, culturally adapt, and validate RoB-SYRCLE for use in experimental studies. Methods: Translation of the tool was performed according to an international guide. After the translation, back-translation, and approval of the version by the creators, the tool, which consists of 10 items in Portuguese, was evaluated by 15 researchers with experience in animal studies. Items 1 and 2 refer to selection bias (sequence generation, baseline

characteristics, and allocation concealment), items 3 and 4 to performance bias, item 5 to execution bias (random housing and blinding), items 6 and 7 to detection bias (random assessment of outcomes and blinding), item 8 to attrition bias (incomplete outcomes), item 9 to reporting bias (report of selective outcome), and item 10 to other sources of bias. The responses of the evaluators addressed their degree of understanding the tool (“I completely understood”, “I partially understood”, or “I did not understand”). Statistical analysis: Descriptive statistics with percentage values. Results: Of the researchers who evaluated the tool, 100%–86.6% fully understood items 1–7, 9, and 10. Item 8 was fully understood by 80% of the researchers. Conclusion: The tool was successfully translated and used by all researchers; however, item 8 may be difficult to understand and apply even in the original language. Furthermore, for item 9, it is necessary to clarify the primary outcome.

Keywords: Translation. Bias. Systematic review.

INTRODUCTION

Many studies in Brazil and worldwide have been carried out using animal experimentation models, and these studies are currently being used in the development of systematic reviews (Hooijmans et al. 2012). Research on animals has grown, leading to an overuse of animals for experimentation. This also led to the emergence of problems of replicability and external validity in pre-clinical research. Preclinical systematic reviews emerged in this context, as a tool to comprehensively analyze scientific publication in experimental animals.

One of the key features systematic reviews is the analysis of risk of bias (Macleod et al. 2009). The assessment of risk of bias in human clinical trials is performed using instruments such as the Cochrane Collaboration Risk of Bias (RoB) Tool (Higgins et al. 2011). An adaptation of this tool was developed by the Systematic Review Center for Laboratory Animal Experimentation (SYRCLE), to assess the risks of scientific methodological bias in animal experimentation (Hooijmans et al. 2014). SYRCLE RoB tool was developed to analyze the risk of scientific bias in experimental animal research, encompassing the following types of bias: selection bias (sequence generation, basal characteristics, and allocation concealment), execution bias (random housing and blinding), detection bias (random assessment of outcomes and blinding), attrition bias (incomplete outcomes), reporting bias (selective outcome reporting), and others (other sources of bias). The questionnaire is intended for qualitative appraisal of data and each item is designed to be answered with the responses YES (for low risk of bias), NO (for high risk of bias), or UNCERTAIN (for when the risk of bias cannot be determined) (Hooijmans et al. 2014).

This tool is only available in the English language. Considering the growing number of systematic review studies in animal models being performed, the translation of this instrument can disseminate and encourage researchers to carry out systematic reviews of preclinical studes. This study aimed to translate the SYRCLE RoB tool into Brazilian Portuguese.

METHODS

Study design

This study was approved by the original authors of the RoB SYRCLE tool. (Hooijmans et al. 2014). The translation of the tool was conducted from May to July 2020. Translation and validation were carried out based on protocols validated by Guillemin et al. 1993, Xie 2018, and Santos 2019, and adapted in five steps.

- Step 1 – In the initial translation phase, two bilingual Brazilian researchers with experience in the RoB SYRCLE tool (GNP and AGB) translated the scale into Brazilian Portuguese.
- Step 2 - In the translation synthesis phase, the translators and two other researchers (TBM and APCFF) prepared a single consensus of the translated scale.
- Step 3 – In the English back-translation phase, two native speakers of English (British, RH and American, ES), who had no previous contact with the original version of the scale, independently translated the consensually approved version in Brazilian Portuguese to English.
- Step 4 - In the consensus version and evaluation phase, the tool re-translated into English was submitted for evaluation by the team that developed the tool (CH and MRH). After this analysis, the tool needed adjustments, which involved a Brazilian researcher (GNP) with experience in systematic reviews in animal models and in the RoB SYRCLE tool, and two bilingual researchers (FLP and APCFF). The version was again translated into Portuguese, back-translated into English, and again sent to the development team for approval.
- Step 5 – Pre-test of the final version: 25 bilingual researchers working with experimental animal models in rats in several fields evaluated the tool translated in relation to the level of understanding of the items (possible answers: fully understand, partially understand, and do not understand).

Statistical analysis

The data were organized in a table in Excel and analyzed using descriptive statistics, and the values were expressed as a percentage.

RESULTS

The translated version of RoB SYRCLE tool into Brazilian Portuguese is described in Table 1.

TABLE 1 – Tool for risk of bias assessment.

Item	Tipo de viés	Domínio	Descrição do domínio	Julgamento pelos autores
1	Viés de seleção	Geração de sequência	Descrever os métodos usados, se houver, para gerar a sequência de alocação em detalhes suficientes para permitir avaliar se ela pode produzir grupos comparáveis.	A sequência de alocação foi gerada e aplicada adequadamente? (*)

2	Viés de seleção	Características basais	Descrever todos os fatores prognósticos ou características dos animais (se houver) que são comparados a fim de julgar se os grupos intervenção e controle eram similares no começo do experimento.	Os grupos eram similares no momento basal ou foram ajustados para confundidores nas análises.
3	Viés de performance	Ocultação da alocação	Descrever o método usado para ocultar a sequência de alocação com detalhes suficientes para determinar se as alocações de intervenção poderiam ter sido previstas antes ou durante a inclusão nos diferentes grupos.	A alocação foi adequadamente ocultada? (*)
4	Viés de performance	Alojamento aleatório	Descrever todas as medidas usadas, se houver, para alojar os animais aleatoriamente na sala.	Os animais foram alojados de forma aleatória durante o experimento?
5	Viés de execução	Cegamento	Descrever todas as medidas, se houver, para cegar cuidadores e pesquisadores de saber qual intervenção cada animal recebeu. Fornecer todas as informações sobre se o cegamento foi efetivo.	Os cuidados e/ou pesquisadores estavam cegados sobre qual intervenção cada animal recebeu durante o experimento?
6	Viés de detecção	Avaliação aleatória dos desfechos	Descrever se os animais foram ou não selecionados aleatoriamente para avaliação dos desfechos e quais métodos para selecionar os animais, se houver, foram utilizados.	Os animais foram selecionados aleatoriamente para avaliação dos desfechos?
7	Viés de detecção	Cegamento	Descrever todas as medidas usadas, se houver, para cegar os avaliadores dos desfechos de saber qual intervenção cada animal recebeu. Fornece informações sobre se o cegamento foi efetivo.	Os avaliadores dos desfechos foram cegados?
8	Viés de atrito	Desfechos incompletos	Descrever se os dados relacionados a cada desfecho principal estão completos, incluindo perdas e exclusões nas análises. Declarar se perdas e exclusões foram relatadas, o número em cada grupo (comparado com o total de animais aleatorizados), razões para perdas e exclusões e quaisquer reinclusões nas análises.	Desfechos com dados incompletos foram adequadamente abordados? (*)
9	Viés de relato	Relato de desfecho seletivo	Indicar como o relato seletivo de desfecho foi examinado e o que foi encontrado.	O estudo está livre de relato seletivo de desfecho? (*)
10	Outro	Outras fontes de viés	Declarar quaisquer preocupações importantes sobre vieses não cobertos por outros domínios desta ferramenta.	O estudo aparentemente está livre de outros problemas que poderiam resultar em um alto risco de viés? (*)

(*): Itens de acordo com os itens da ferramenta de risco de viés da C

PERGUNTAS GUIA

As perguntas-guia (PG) adicionais são incluídas para auxiliar a avaliação. “Sim” indica baixo risco de viés; Não” indica alto risco de viés; “Incerto” indica risco de viés incerto. Se uma das perguntas é respondida com “Não”, isto indica alto risco de viés para aquele tópico específico.

1. A sequência de alocação foi gerada e aplicada adequadamente?
    - PG 1.1: Os pesquisadores descreveram um componente aleatório no processo de geração de sequência e alocação dos animais em diferentes grupos? (Sim / Não / Incerto)
        - Referência a uma tabela de números aleatórios.
        - Uso de um gerador computacional de números aleatórios.
    - Informações adicionais:
        - Exemplos de abordagem não aleatória.
            - Alocação por julgamento ou preferência do pesquisador.
            - Alocação baseada nos resultados de testes de laboratório ou de uma série de testes.
            - Alocação por disponibilidade de intervenção.
            - Sequência gerada por data de nascimento par ou ímpar.
            - Sequência gerada por alguma regra baseada no número do animal ou da caixa.

2. Os grupos eram similares no momento basal ou foram ajustados para confundidores nas análises?
    - PG 2.1: A distribuição das características relevantes foi balanceada para os grupos intervenção e controle? (Sim / Não / Incerto).
    - PG 2.2: Se relevante, os investigadores ajustaram adequadamente as análises para distribuição desigual de alguma característica basal importante? (Sim / Não / Incerto).
    - PG 2.3: O momento de indução da doença foi adequado? (Sim / Não / Incerto).
    - Informações adicionais
        - O número e tipo das características bases são dependentes da pergunta da revisão. Portanto, antes de iniciar a avaliação de risco de viés os revisores devem discutir quais características basais precisam ser comparáveis entre os grupos. Por exemplo, em um RS que investiga os efeitos da hipotermia no tamanho do infarto, deveriam ser similares entre os grupos no início do estudo as seguintes variáveis: distribuição de gênero, peso do ventrículo esquerdo, frequência cardíaca e pressão arterial.
        - A descrição das características basais e/ou confundidores geralmente contém:
            - O sexo, idade e peso dos animais.
            - Valores basais dos desfechos de interesse do estudo.
        - Momento de indução da doença: Em alguns estudos de prevenção, a doença é induzida depois da alocação da intervenção. Por exemplo,

em um experimento com suplementação preventiva de probioóticos em pancreatite aguda, a pancreatite é induzida depois da alocação dos animais para o grupo que receberá probiótico ou para o grupo controle. Para reduzir desequilíbrios em medidas basais, o momento da indução da doença deve ser igual para ambos os grupos de tratamento. Exemplos de momento de indução de doença adequados:

- A doença foi induzida antes da aleatorização da intervenção.
- A doença foi induzida depois da aleatorização da intervenção, mas o momento da indução da doença foi aleatorizado, e a pessoa responsável pela indução da doença foi adequadamente cegado sobre qual intervenção cada animal receberia.

3. A alocação em diferentes grupos foi adequadamente ocultada durante o experimento?
    - PG 3.1: O pesquisador responsável por alocar os animais ao grupo intervenção ou ao grupo controle não poderia prever a atribuição aos grupos devido a um dos seguintes métodos ou a métodos equivalentes? (Sim / Não / Incerto)
        - Codificação da alocação em grupo experimental e grupo controle por uma terceira parte.
        - Aleatorização realizada por uma terceira parte.
        - Uso de envelopes opacos, selados, numerados sequencialmente.
    - Informações adicionais:
        - Exemplos em que os pesquisadores possivelmente poderiam prever a atribuição aos grupos:
            - Cronograma de aleatorização aberto.
            - Envelopes sem proteção adequada.
            - Alternância ou rotação.
            - Alocação baseada na data de nascimento.
            - Alocação baseada no número do animal.
            - Qualquer outro procedimento explicitamente não ocultado de abordagem não aleatória.

4. Os animais foram alojados aleatoriamente durante o experimento?
    - PG 4.1: Os pesquisadores dispuseram as caixas, gaiolas ou animais de maneira aleatória no local onde os animais são mantidos (biotério/sala de animais/vivário)? (Sim / Não / Incerto).
        - Animais foram selecionados aleatoriamente durante a avaliação do desfecho (usar perguntas-guia do item 6).
    - PG 4.2: É improvável que o desfecho ou a avaliação do desfecho tenha sido influenciada pelo alojamento não aleatório dos animais? (Sim / Não / Incerto).
        - Os animais de diferentes grupos experimentais vivem juntos em uma mesma gaiola/pasto (ex.: condições de alojamento são idênticas).
    - Informações adicionais.
        - Exemplos de pesquisadores que usam abordagem não aleatório para dispor as gaiolas/caixas.
            - Grupos experimentais foram estudados em locais diferentes (ex.: grupo A no laboratório A ou na prateleira A; grupo B no laboratório B ou na prateleira B).

5. Os cuidadores e/ou investigadores estavam cegados sobre qual intervenção cada animal recebeu durante o experimento?
    - PG 5.1: O cegamento dos cuidadores e investigadores foi assegurado e foi improvável que esse cegamento tenha sido quebrado? (Sim / Não / Incerto).
        - Os cartões de identificação dos animais e/ou das gaiolas foram codificados e têm aparência idênticas.
        - Frascos de drogas/medicamentos identificados sequencialmente são têm aparência idêntica.
        - As circunstâncias durante a intervenção são especificadas e semelhantes em ambos os grupos.
        - As condições de alojamento dos animais durante o experimento são aleatorizadas dentro da sala (use os critérios do item 4).
    - Informações adicionais
        - Exemplos de cegamento inapropriados
            - Etiquetas ou rótulos coloridos nas gaiolas (ex..: vermelho para o grupo A, amarelo para o grupo B).
            - As condições de alojamento dos animais não são randomizadas na sala durante o experimento (use os critérios do item 4).
            - Diferenças esperadas em efeitos visíveis entre grupos experimental e controle.
            - O indivíduo que prepara o experimento é o mesmo que conduz e analisa o experimento.
            - Circunstâncias durante a intervenção não são semelhantes em ambos os grupos.
        - Exemplos em que circunstâncias durante a intervenção não foram semelhantes:
            - Momento da administração do placebo e da droga experimental foi diferente.
            - Instrumentos usados para realizar os experimentos diferem entre os grupos experimental e controle (ex..: experimentos sobre efeitos da pressão abdominal, em que grupo experimental recebe cirurgia e agulha para aumentar pressão, enquanto o grupo controle recebe apenas cirurgia).
            - **A relevância dos itens mencionados acima depende dos experimentos. Os revisores precisam julgar quais dos itens acima mencionados poderiam causar viés nos resultados quando desiguais. Esses devem ser avaliados.

6. Os animais foram selecionados aleatoriamente para avaliação dos desfechos?
    - PG 6.1: Os investigadores selecionaram aleatoriamente um animal durante avaliação do desfecho, ou usaram um componente aleatorizado na geração de sequência para avaliação do desfecho? (Sim / Não / Incerto).
        - Exemplos de geração de sequência aleatória para avaliação de desfecho:
            - Referência a tabela de números aleatórios.
            - Uso de um programa de computador para gerar números aleatórios.

7. O avaliador de desfechos foi cegado?

- PG 7.1: O cegamento do avaliador dos desfechos foi garantido e era improvável que o cegamento pudesse ter sido quebrado? (Sim / Não / Incerto)
    - Os métodos de avaliação do desfecho foram os mesmos nos dois grupos.
    - Os animais foram selecionados aleatoriamente durante a avaliação dos desfechos (usar perguntas-guia do item 6).
- "O avaliador do desfecho não foi cegado, mas os revisores julgam que não é provável que o desfecho seja influenciado pela falta de cegamento? (ex..: mortalidade)" (Sim / Não / Incerto).
- Informações adicionais:
    - Este item precisa ser avaliado para cada desfecho principal.

8. Os desfechos com dados incompletos foram adequadamente abordados?
    - PG 8.1: Todos os animais foram incluídos nas análises? (Sim / Não/ Incerto).
    - PG 8.2: As razões para falta de dados não provavelmente não estavam relacionadas ao desfecho real (ex.: falha técnica)? (Sim / Não / Incerto).
    - PG 8.3: Os dados de desfecho faltantes estão equilibrados entre os grupos, com razões semelhantes para a falta de dados entre os grupos? (Sim / Não / Incerto).
    - PG 8.4: A imputação de dados faltantes foi feita utilizando métodos apropriados? (Sim / Não / Incerto).

9. O estudo está livre de relato de desfecho seletivo?
    - PG 9.1: O protocolo do estudo estava disponível e todos os desfechos primários e secundários pré-específicados do estudo foram relatados no manuscrito atual? (Sim / Não / Incerto).
    - PG 9.2: O protocolo do estudo não estava disponível, mas ficou claro que o relato publicado incluiu todos os desfechos esperados (ou seja, comparando-se as seções de métodos e resultados)? (Sim / Não / Incerto).
    - Informações adicionais:
        - Relato seletivo de desfechos:
            - Nem todos os desfechos primários pré-especificados foram relatados.
            - Um ou mais desfechos primários foram relatados usando medidas, métodos de análise ou subconjuntos de dados (ex.: sub-escalas) que não foram pré-especificadas no protocolo.
            - Um ou mais desfechos primários não foram pré-especificados (a menos que clara justificativa para o relato dado – ex.: efeitos adversos inesperados).
            - O relato do estudo falhar em incluir resultados de um desfecho-chave que seria esperado relatar para tal estudo.

10. O estudo aparentemente está livre de outros problemas que poderiam resultar em alto risco de viés?
    - PG 10.1: O estudo foi livre de contaminação (combinação de drogas)? (Sim / Não / Incerto).
    - PG 10.2: O estudo foi livre de influência inapropriada de financiadores ou patrocinadores? (Sim / Não / Incerto).

- PG 10.3: O estudo foi livre de erros nas unidades de análises (unidades experimentais)? (Sim / Não / Incerto).
- PG 10.4: Riscos de viés específicos ao desenho experimental estavam ausentes? (Sim / Não / Incerto).
- PG 10.5: Novos animais foram adicionados ao grupo controle e experimental para substituir perdas da população original? (Sim / Não / Incerto).
- Informações adicionais
    - A relevância das perguntas-sinalizadoras (Tabela 3) depende do experimento. Os revisores precisam julgar por si próprios quais itens podem causar viés nos resultados e devem ser avaliados.
    - Contaminação / combinação de drogas
        - Experimentos nos quais os animais recebem, além da droga de intervenção – tratamento adicional ou drogas que podem influenciar ou causar viés no resultado.
    - Erros na unidade de análise:
        - Intervenções em diferentes partes do corpo em um mesmo participante (ex.: um olho experimental, outro olho controle).
        - Todos os animais recebendo a mesma intervenção estão na mesma gaiola, mas a análise é conduzida como se cada animal fosse uma única unidade experimental.
    - Vieses específicos ao desenho experimental:
        - Desenho cruzado (crossover) inadequado (intervenção sem efeito temporário ou doença não estável ao longo do tempo).
        - Desenho cruzado (crossover) com risco de efeito residual (carry-over effect).
        - Desenho cruzado (crossover) com apenas dados do primeiro período disponível.
        - Desenho cruzado (crossover) em que muitos animais não recebem o segundo tratamento (ou o tratamento seguinte) devido ao alto número de perdas ou pela duração do estudo.
        - Desenho cruzado (crossover) em que todos os animais recebem a mesma ordem de intervenções.
        - Estudo de braços múltiplos em que as mesmas comparações de grupos não são relatadas para todos os desfechos (relato de desfecho seletivo).
        - Estudo de braços múltiplos em que diferentes braços são combinados (todos os dados deveriam ser apresentados por grupo).
        - Ensaio randomizado por cluster não levando em conta o agrupamento durante as análises estatísticas (erro de unidade experimental).
        - Desenho cruzado (crossover) em que a análise pareada dos resultados não é levada em consideração.

Figure 1 shows the percentage of responses regarding the understanding of the 25 researchers who evaluated the translated tool.

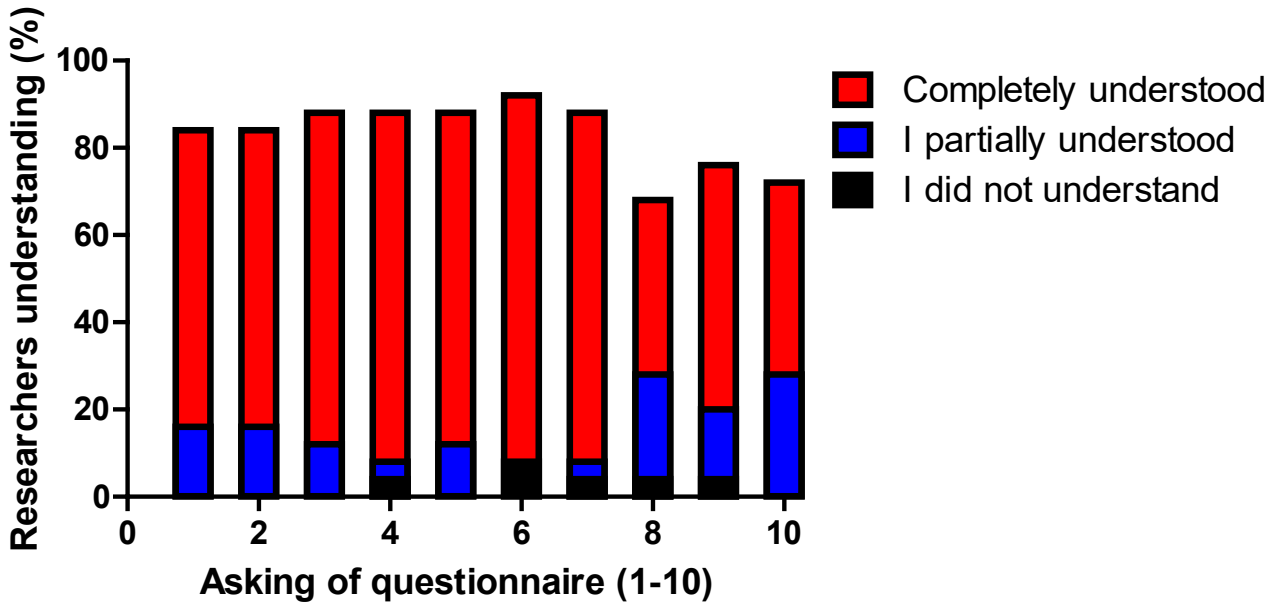

Figure 1. The x-axis represents questions 1–10, and the y-axis the percentage of understanding for each question.

Some uncertainties were reported by the evaluators. The description of the possible answers was questioned. For the translated version, they suggested a better explanation of the answers ‘uncertain’ and ‘no high risk of bias’. The researchers were unsure as to whether ‘uncertain’ could be applied to when a finding was not reported in the studies and whether findings not being reported could be considered a high risk of bias.

Regarding question 2 (Selection Bias – Baseline Characteristics), the use of keywords that normally describe certain situations in English was suggested.

For question 8, Attrition Bias - Incomplete Outcomes, the reader was asked, as an example, to determine the number of animals described in the methods and to check in the tables and legends whether all the animals were used for the analyses. The terms “reinclusion for analysis” and “incomplete outcome” were pointed out as being confusing.

In the item Reporting Bias (question 9) - Selective Outcome report, the evaluator did not understand that there were two possible answers.

DISCUSSION

This study aimed to translate the RoB SYRCLE tool into Portuguese. The method used in the present study enabled translation for later cross-cultural adaptation and validation of RoB SYRCLE tool for Brazilian culture. When validated, the questionnaire will be able to assess the risk of methodological bias in experimental research and thus provide researchers in Brazil with greater ease in assessing the methodological quality of studies that will be included in systematic reviews of animal studies.

After the initial steps and translation back to the original language, the creators evaluated it, a new translation with adjustments was performed. To contact the authors, we collaborated with a member of the Brazilian Reproducibility Initiative in Preclinical Systematic Review and Meta-Analysis, a group of researchers that aims to increase the development of systematic reviews and meta-analyses in animals. In our study, the semantic, idiomatic, and grammatical equivalences of some items were necessary, consistent with the study reported by Santos et al. 2020, which, in order to guarantee the meaning of the original language, maintained semantic equivalence.

The researchers had a good understanding of most of the tool; however, questions 8 and 9, due to understanding not reaching an 80% level, requires adjustments for Portuguese. Some suggestions, such as clarifying “primary outcomes” and how to address a lack of outcome, were made to increase clarity. In the original tool, the answer "yes" indicates a low risk of bias, “no” indicates a high risk of bias, and “uncertain” indicates an uncertain risk of bias. The evaluators mostly commented that the three options confused them, but mainly reported having doubts about marking “uncertain” and “no high risk of bias”, and stated that “the uncertain term should be better clarified as to your use.” They left questions such as “should “uncertain” be used only when it was done? Why was it not explained?” Another question that also needed clarification was “if some items of the tool seem to not apply to some articles, more items in the article such as “does not apply” should be added”. Other translation studies went through similar processes in which adaptations of the questionnaires were necessary according to the region in which they will be applied (Claro et al. 2011; Pereira et al. 2011), which reinforces our findings.

The evaluators suggested some modifications regarding the judgment of each item of the questionnaire; therefore, the validation process of the Brazilian Portuguese version of the RoB SYRCLE tool will continue from the translated version reported in this study.

Conclusion

The tool was successfully translated and used by all researchers; only items 8 and 9 required greater attention due to the percentage of understanding not reaching 80%.

However, item 8 can be difficult to understand and apply even in the original language. In contrast, in item 9, it is necessary to clarify the primary outcome.

References

Carlijn R Hooijmans1, Maroeska M Rovers, Rob BM de Vries, Marlies Leenaars, Merel Ritskes-Hoitinga and Miranda W Langendam. SYRCLE's risk of bias tool for animal studies. BMC Medical Research Methodology 2014, 14:43.

Claro, H. G., Oliveira, M. A. F. de, Almeida, M. M. de, Vargas, D. de, & Plaglione, H. B. (2011). Adaptação cultural de instrumentos de coleta de dados para mensuração em álcool e drogas. SMAD, Revista Eletrônica Saúde Mental Álcool E Drogas (Edição Em Português), 7(2), 71-77. https://doi.org/10.11606/issn.1806-6976.v7i2p71-77

Groenwold RH. 3 vormen van bias: vertekening van onderzoeksresultaten en hoe dat te voorkomen [Three types of bias: distortion of research results and how that can be prevented]. Ned Tijdschr Geneeskd. 2013;157(40):A6497.
Guillemin F, Bombardier B and Beaton D. Cross-cultural adaptation of health-related quality of life measures: literature review and proposed guidelines. J Clio itpidemiol. 1993; 46(12):1417-1432.

Higgins J P T, Altman D G, Gøtzsche P C, Jüni P, Moher D, Oxman A D et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials BMJ 2011; 343 :d5928 doi:10.1136/bmj.d5928

Hooijmans CR, Rovers M, de Vries RB, Leenaars M, Ritskes-Hoitinga M: An initiative to facilitate well-informed decision-making in laboratory animal research: report of the First International Symposium on Systematic Reviews in Laboratory Animal Science. Lab Anim 2012, 46(4):356–357.

Hooijmans CR, Tillema A, Leenaars M, Ritskes-Hoitinga M. Enhancing search efficiency by means of a search filter for finding all studies on animal experimentation in PubMed. Lab Anim. 2010;44(3):170-5.

Macleod MR, Fisher M, O'Collins V, Sena ES, Dirnagl U, Bath PM, Buchan A, van der Worp HB, Traystman R, Minematsu K, Donnan GA, Howells DW: Good laboratory practice: preventing introduction of bias at the bench. Stroke 2009, 40(3):e50–e52.

Pereira, Vanessa Santos et al. Tradução e validação para a língua portuguesa de um questionário para avaliação da gravidade da incontinência urinária. Revista Brasileira de

Ginecologia e Obstetrícia. 2011,33 -4, pp. 182-187. 2011. ISSN 1806-9339. https://doi.org/10.1590/S0100-72032011000400006.

Santos DHN dos, Soares JFR, da Nóbrega AC e Pernambuco UL et al. Tradução e adaptação transcultural do Thyroidectomy-Related Voice Questionnaire (TVQ) para o português brasileiro. CoDAS 2020, 32, 5 e20190150. https://doi.org/10.1590/2317-1782/20202019150.

Santos RZ, Ghisi GLM, Bonin CDB, Chaves G, Haase CM, Britto RR et al. Validation of the Brazilian Version of CADE-Q II to Assess Knowledge of Coronary Artery Disease Patients. Arq. Bras. Cardiol. 2019 ; 112(1): 78-84.

Xie, G., Chen, L., Yang, S. et al. Simplified Chinese translation of 13 adult item banks from the Quality of Life in Neurological Disorders (Neuro-QoL). BMC Health Serv Res 18, 825 (2018). https://doi.org/10.1186/s12913-018-3631-7.

ANEXO 3 - NORMAS DA REVISTA BMC MEDICAL RESEARCH METHODOLOGY

Preparing your manuscript

This section provides general style and formatting information only. Formatting guidelines for specific article types can be found below.

Research article

Database article

Software article

Review

General formatting guidelines

Preparing main manuscript text

Preparing illustrations and figures

Preparing tables

Preparing additional files

Preparing figures

Back to top

When preparing figures, please follow the formatting instructions below.

Figures should be numbered in the order they are first mentioned in the text, and uploaded in this order. Multi-panel figures (those with parts a, b, c, d etc.) should be submitted as a single composite file that contains all parts of the figure.

Figures should be uploaded in the correct orientation.

Figure titles (max 15 words) and legends (max 300 words) should be provided in the main manuscript, not in the graphic file.

Figure keys should be incorporated into the graphic, not into the legend of the figure.

Each figure should be closely cropped to minimize the amount of white space surrounding the illustration. Cropping figures improves accuracy when placing the figure in combination with other elements when the accepted manuscript is prepared for publication on our site. For more information on individual figure file formats, see our detailed instructions.

Individual figure files should not exceed 10 MB. If a suitable format is chosen, this file size is adequate for extremely high quality figures.

Please note that it is the responsibility of the author(s) to obtain permission from the copyright holder to reproduce figures (or tables) that have previously been published elsewhere. In order for all figures to be open access, authors must have permission from the rights holder if they wish to include images that have been published elsewhere in non open access journals. Permission should be indicated in the figure legend, and the original source included in the reference list.

Figure file types

We accept the following file formats for figures:

EPS (suitable for diagrams and/or images)

PDF (suitable for diagrams and/or images)

Microsoft Word (suitable for diagrams and/or images, figures must be a single page)

PowerPoint (suitable for diagrams and/or images, figures must be a single page)

TIFF (suitable for images)

JPEG (suitable for photographic images, less suitable for graphical images)

PNG (suitable for images)

BMP (suitable for images)

CDX (ChemDraw - suitable for molecular structures)

For information and suggestions of suitable file formats for specific figure types, please see our author academy.

Figure size and resolution

Figures are resized during publication of the final full text and PDF versions to conform to the BioMed Central standard dimensions, which are detailed below.

Figures on the web:

width of 600 pixels (standard), 1200 pixels (high resolution).

Figures in the final PDF version:

width of 85 mm for half page width figure

width of 170 mm for full page width figure

maximum height of 225 mm for figure and legend

image resolution of approximately 300 dpi (dots per inch) at the final size

Figures should be designed such that all information, including text, is legible at these dimensions. All lines should be wider than 0.25 pt when constrained to standard figure widths. All fonts must be embedded.

Figure file compression

Vector figures should if possible be submitted as PDF files, which are usually more compact than EPS files.

TIFF files should be saved with LZW compression, which is lossless (decreases file size without decreasing quality) in order to minimize upload time.

JPEG files should be saved at maximum quality.

Conversion of images between file types (especially lossy formats such as JPEG) should be kept to a minimum to avoid degradation of quality.

If you have any questions or are experiencing a problem with figures, please contact the customer service team at info@biomedcentral.com.

Preparing main manuscript text

Back to top

Quick points:

Use double line spacing

Include line and page numbering

Use SI units: Please ensure that all special characters used are embedded in the text, otherwise they will be lost during conversion to PDF

Do not use page breaks in your manuscript

File formats

The following word processor file formats are acceptable for the main manuscript document:

Microsoft word (DOC, DOCX)

Rich text format (RTF)

TeX/LaTeX (use BioMed Central's TeX template)

Please note: editable files are required for processing in production. If your manuscript contains any non-editable files (such as PDFs) you will be required to re-submit an editable file when you submit your revised manuscript, or after editorial acceptance in case no revision is necessary.

Additional information for TeX/LaTeX users

Please use BioMed Central's TeX template and BibTeX stylefile if you use TeX format. Submit your references using either a bib or bbl file. When submitting TeX submissions, please submit both your TeX file and your bib/bbl file as manuscript files. Please also convert your TeX file into a PDF (please do not use a DIV file) and submit this PDF as a supplementary file with the name 'Reference PDF'. This PDF will be used by our production team as a reference point to check the layout of the article as the author intended.

The Editorial Manager system checks for any errors in the Tex files. If an error is present then the system PDF will display LaTex code and highlight and explain the error in a section beginning with an exclamation mark (!).

All relevant editable source files must be uploaded during the submission process. Failing to submit these source files will cause unnecessary delays in the production process.

TeX templates

BioMedCentral_article (ZIP format) - preferred template

article (part of the standard TeX distribution)

amsart (part of the standard TeX distribution)

Style and language

For editors and reviewers to accurately assess the work presented in your manuscript you need to ensure the English language is of sufficient quality to be understood. If you need help with writing in English you should consider:

Getting a fast, free online grammar check.

Visiting the English language tutorial which covers the common mistakes when writing in English.

Asking a colleague who is proficient in English to review your manuscript for clarity.

Using a professional language editing service where editors will improve the English to ensure that your meaning is clear and identify problems that require your review. Two such services are provided by our affiliates Nature Research Editing Service and American Journal Experts. BMC authors are entitled to a 10% discount on their first submission to either of these services. To claim 10% off English editing from Nature Research Editing Service, click here. To claim 10% off American Journal Experts, click here.

Please note that the use of a language editing service is not a requirement for publication in the journal and does not imply or guarantee that the article will be selected for peer review or accepted.

Data and materials

For all journals, BioMed Central strongly encourages all datasets on which the conclusions of the manuscript rely to be either deposited in publicly available repositories (where available and appropriate) or presented in the main paper or additional supporting files, in machine-readable format (such as spread sheets rather than PDFs) whenever possible. Please see the list of recommended repositories in our editorial policies.

For some journals, deposition of the data on which the conclusions of the manuscript rely is an absolute requirement. Please check the Instructions for Authors for the relevant journal and article type for journal specific policies.

For all manuscripts, information about data availability should be detailed in an ‘Availability of data and materials’ section. For more information on the content of this section, please see the Declarations section of the relevant journal’s Instruction for Authors. For more information on BioMed Centrals policies on data availability, please see our [editorial policies].

Formatting the 'Availability of data and materials' section of your manuscript

The following format for the 'Availability of data and materials section of your manuscript should be used:

"The dataset(s) supporting the conclusions of this article is(are) available in the [repository name] repository, [unique persistent identifier and hyperlink to dataset(s) in http:// format]."

The following format is required when data are included as additional files:
"The dataset(s) supporting the conclusions of this article is(are) included within the article (and its additional file(s))."
BioMed Central endorses the Force 11 Data Citation Principles and requires that all publicly available datasets be fully referenced in the reference list with an accession number or unique identifier such as a DOI.
For databases, this section should state the web/ftp address at which the database is available and any restrictions to its use by non-academics.
For software, this section should include:
Project name: e.g. My bioinformatics project
Project home page: e.g. http://sourceforge.net/projects/mged
Archived version: DOI or unique identifier of archived software or code in repository (e.g. enodo)
Operating system(s): e.g. Platform independent
Programming language: e.g. Java
Other requirements: e.g. Java 1.3.1 or higher, Tomcat 4.0 or higher
License: e.g. GNU GPL, FreeBSD etc.
Any restrictions to use by non-academics: e.g. licence needed
Information on available repositories for other types of scientific data, including clinical data, can be found in our editorial policies.
References
See our editorial policies for author guidance on good citation practice.

Please check the submission guidelines for the relevant journal and article type.
What should be cited?
Only articles, clinical trial registration records and abstracts that have been published or are in press, or are available through public e-print/preprint servers, may be cited.
Unpublished abstracts, unpublished data and personal communications should not be included in the reference list, but may be included in the text and referred to as "unpublished observations" or "personal communications" giving the names of the involved researchers. Obtaining permission to quote personal communications and unpublished data from the cited colleagues is the responsibility of the author. Only footnotes are permitted. Journal abbreviations follow Index Medicus/MEDLINE.
Any in press articles cited within the references and necessary for the reviewers' assessment of the manuscript should be made available if requested by the editorial office.
How to format your references

Please check the Instructions for Authors for the relevant journal and article type for examples of the relevant reference style.

Web links and URLs: All web links and URLs, including links to the authors' own websites, should be given a reference number and included in the reference list rather than within the text of the manuscript. They should be provided in full, including both the title of the site and the URL, as well as the date the site was accessed, in the following format: The Mouse Tumor Biology Database. http://tumor.informatics.jax.org/mtbwi/index.do. Accessed 20 May 2013. If an author or group of authors can clearly be associated with a web link, such as for weblogs, then they should be included in the reference.

Authors may wish to make use of reference management software to ensure that reference lists are correctly formatted.

Preparing tables

Back to top

When preparing tables, please follow the formatting instructions below.

Tables should be numbered and cited in the text in sequence using Arabic numerals (i.e. Table 1, Table 2 etc.).

Tables less than one A4 or Letter page in length can be placed in the appropriate location within the manuscript.

Tables larger than one A4 or Letter page in length can be placed at the end of the document text file. Please cite and indicate where the table should appear at the relevant location in the text file so that the table can be added in the correct place during production.

Larger datasets, or tables too wide for A4 or Letter landscape page can be uploaded as additional files. Please see [below] for more information.

Tabular data provided as additional files can be uploaded as an Excel spreadsheet (.xls) or comma separated values (.csv). Please use the standard file extensions.

Table titles (max 15 words) should be included above the table, and legends (max 300 words) should be included underneath the table.

Tables should not be embedded as figures or spreadsheet files, but should be formatted using ‘Table object’ function in your word processing program.

Color and shading may not be used. Parts of the table can be highlighted using superscript, numbering, lettering, symbols or bold text, the meaning of which should be explained in a table legend.

Commas should not be used to indicate numerical values.

If you have any questions or are experiencing a problem with tables, please contact the customer service team at info@biomedcentral.com.

Preparing additional files

Back to top

As the length and quantity of data is not restricted for many article types, authors can provide datasets, tables, movies, or other information as additional files.

All Additional files will be published along with the accepted article. Do not include files such as patient consent forms, certificates of language editing, or revised versions of the main manuscript document with tracked changes. Such files, if requested, should be sent by email to the journal's editorial email address, quoting the manuscript reference number. Please do not send completed patient consent forms unless requested.

Results that would otherwise be indicated as "data not shown" should be included as additional files. Since many web links and URLs rapidly become broken, BioMed Central requires that supporting data are included as additional files, or deposited in a recognized repository. Please do not link to data on a personal/departmental website. Do not include any individual participant details. The maximum file size for additional files is 20 MB each, and files will be virus-scanned on submission. Each additional file should be cited in sequence within the main body of text.

If additional material is provided, please list the following information in a separate section of the manuscript text:

File name (e.g. Additional file 1)

File format including the correct file extension for example .pdf, .xls, .txt, .pptx (including name and a URL of an appropriate viewer if format is unusual)

Title of data

Description of data

Additional files should be named "Additional file 1" and so on and should be referenced explicitly by file name within the body of the article, e.g. 'An additional movie file shows this in more detail [see Additional file 1]'.

For further guidance on how to use Additional files or recommendations on how to present particular types of data or information, please see How to use additional files.