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ANÁLISIS METABOLÓMICO Y TRANSCRIPTÓMICO DIFERENCIAL DE PACIENTES PREDIABÉTICOS, DIABÉTICOS Y CON NEFROPATÍA DIABÉTICA PARA IDENTIFICAR POTENCIALES BIOMARCADORES DE DAÑO RENAL

TESIS QUE PRESENTA

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PARA OBTENER EL GRADO DE:
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Análisis metabolómico y transcriptómico diferencial de pacientes prediabéticos, diabéticos y con nefropatía diabética para identificar potenciales biomarcadores de daño renal

by Juan José Oropeza Valdez

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Transcriptome analysis identifies oxidative stress injury biomarkers for Diabetic Nephropathy

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Abstract

Background. The early diagnosis of diabetic nephropathy (DN) is essential to improve the prognosis and manage patients affected by this disease. Standard biomarkers, including albuminuria and glomerular filtration rate, are limited to give a precise result. New molecular biomarkers are needed to identify better and predict DN disease evolution. Characteristic DN biomarkers can be identified using transcriptomic analysis.

Aim of the study. To evaluate the transcriptomic profile of controls (CTRLs, n = 15), prediabetes (PREDM, n = 15),, type-2 diabetes mellitus (DM-2, n = 15), and DN (n = 15) patients by microarray analysis to find new biomarkers, RT-PCR was used to confirm gene biomarkers specific for DN.

Materials and methods. Blood samples were used to isolate RNA for microarray expression microarrays evaluating 26,803 unique gene sequences and 30,606 LncRNA sequences, selected gene biomarkers for DN were validated using qPCR assays. Sensitivity, specificity, and area under the curve (AUC) were calculated as measures of diagnostic accuracy.

Results. The DN transcriptome, founding here, were composed by 300 induced genes, compared to CTRLs, PREDM, and DM-2 groups. RT-qPCR assays validated that METLL22, PFKL , CCNB1 and CASP2 genes were induced in the DN group compared to CTRLs, PREDM, and DM-2 groups. The ROC analysis for these four genes showed 0.9719, 0.8853, 0.8533 and 0.7748 AUC values respectively.

Conclusion. Among induced genes in the DN group, we found that CASP2, PFKL and CCNB1 can be used as potential biomarkers to diagnose DN, where, METLL22 represents the best with an AUC=0.9719.

Keywords: diabetic nephropathy; diabetes; transcriptome; microarray; biomarkers.

Introduction

Diabetic nephropathy (DN), also referred as diabetic renal disorder or diabetic kidney disease, is a common and severe microvascular complication of type 2 diabetes (DM-2), which evolves to an end-stage renal disorder (ESRD) if not properly detected and treated. With the increasing incidence of DM-2 worldwide, DN has become a health concern[1], since approximately 40% of DM-2 patients develop DN. The effective treatment of DN requires glycemic control and antihypertensive measures[2], and is more effective if starts as promptly as DN is detected. Thus, it is of pivotal importance the diagnosis of DN during its early stages[3].

The glomerular filtration rate (GFR) is the main biomarker used for predicting ESRD or DN in both clinical practice and trials. The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) and Modification of Diet in Renal Disease (MDRD) equations, both based on serum creatinine, are commonly used to estimate GFR. Colhoun et al. [4] showed that the accuracy for both equations is low, ranging from 65% to 69%, and as discussed by Porrini et al[5], these equations have underlying errors in the use of creatinine for the estimation of GFR. By the other hand, microalbuminuria is another commonly investigated biomarker for the diagnosis of DN. However, its diagnostic value in early-stage DN is limited when renal injury commonly precedes proteinuria, as is described by Perkins et al., who showed that only 52% of patients with advanced-stage DN developed proteinuria during a 12-year follow-up study. In another study [6], it was found that only 33% of patients with type 1 diabetes with confirmed microalbuminuria developed the clinically apparent renal disorder. Other studies have reaffirmed these findings, demonstrating that a substantial portion of diabetic patients with renal dysfunction do not have proteinuria [7-9].

Omics technologies are high-throughput techniques that allow the collection, in a single experiment, of large amounts of data, such as an entire library of genes and their signature expression, which can be used to identify biomarkers. Bioinformatics tools have enabled the extensive analysis and interpretation of such massive data. It has been well-established that glomerular damage, tubular injury, inflammatory responses, and oxidative stress contribute to the process of developing diabetic kidney disease[10]. Since all these

processes are complex and difficult to tackle without a strategy that contemplates global changes at multiple levels, we propose the use of omics to find a transcriptome signature able to identify patients in the early stages of diabetes in progress to developing DN. In this study, we were able to identify four blood biomarkers involved in pathological pathways (glomerular damage, mesangial expansion, oxidative stress, and metabolic dysfunction) using transcriptional analysis with mRNA microarrays and qPCR. Given that these processes occur in prediabetic or diabetic groups, as well as in patients with DN, we suggest that the use as surrogate biomarkers may have a potential application in the diagnosis of early-stage DN.

Materials and Methods

Study population

A total of 60 subjects (fifteen per group) were recruited from Zacatecas, Mexico, from November 2015 to December 2018. Laboratory analysis for fasting glucose, hemoglycated hemoglobin, creatinine, urea, general urine test, 24-hour creatinine clearance (ml/min/1.72m^2), microalbuminuria, and urine proteins for all patients was done. All adult patients were classified using the American Diabetes Association classification based on hemoglycated hemoglobin (HbA1c): Controls (CTRLS) (HbA1c <5.6%), Prediabetes (PREDM) (HbA1c 5.7 to 6.3%), type 2 diabetes (DM-2) (HbA1c> 6.4%). Diabetic Patients with diabetic nephropathy were referred from the Nephrology consultation. Patients under follow-up by the HGZ1 service were classified after assessing renal function according to the Mogensen and KDIGO classification with results of blood chemistry, creatinine clearance, proteinuria and albuminuria in urine for 24 hrs, patients with urinary tract infection were ruled out as well as patients who could have other added kidney disease Table 1 shows general demographic and clinical data from patient. The study was approved by the IMSS National Committee for Scientific Research and Ethics (Registry R-2017-785-131) and follows the international ethical standards of the Helsinki convention for research studies in humans[36]. All participants signed an informed consent letter.

Sample's processing.

Four mL of peripheral blood from each participant were taken and homogenized in vacutainer-EDTA tubes (Becton-Dickinson,USA) . One mL of RNA later (Thermo Fisher

Scientific, USA) was added immediately after blood collection to maintain the integrity of the RNA and stored at -70 °C until use. Blood and urine samples were taken from each participant to perform laboratory tests for kidney function.

RNA isolation.

Frozen blood samples were processed by combining the conventional Trizol-Chloroform method (Invitrogen, USA) with the QIAmp column protocol (Qiagen Inc, USA), following the manufacturer's instructions. RNA concentration and purity were determined using an ND-1000 nano-droplet kit (Thermo Fisher Scientific, USA). The RNA integrity number (RIN) was determined using the RNA 6,000 Nano kit (Agilent Technologies, USA), with a Bioanalyzer-2100 instrument (Agilent Technologies Genomics, USA) according to the manufacturer instructions. Microarrays were made using only RNA samples with 260/280 index > 2 and RIN> 6.

Expression microarray assays.

Six samples of cRNA from each group of patients were used (a total of 24). Two hundred ng of total RNA per sample was processed according to the "Single Color Microarray Based Gene Expression Analysis Protocol" (Agilent Technologies, USA). According to the supplier's instructions, each of the fragmented cRNA samples was hybridized with the high human density AS G4851C 8X60K microarray (Agilent Technologies, USA) for 17 hours at 65 °C. These microarrays contain 26,803 unique gene sequences and 30,606 LncRNA sequences. Image subtractions were performed using the G4900DA "SureScan Microarray Scanner" laser reader (Agilent Technologies, USA). Mean fluorescence intensity (MFI) values for each sequence were assigned using the "Agilent Feature Extraction" program (Agilent Technologies, USA).

Statistical analysis (microarrays).

Unsupervised data analysis was generated from each sample that met the hybridization quality criteria (splash controls, Q.C. report, etc.). Logarithmic normalization on expression values was carried out. The fold-change on induced or repressed genes (in the groups, it was determined using a "moderate t-test" (empirical Bayes). A heat-map was made with the gene expression values "aver exp" that had a p value <0,05. Data were processed with the Bioconductor environment using R Studio's statistical program (R Foundation for

Statistical Computing, 2016), applying the Limma [11] package. Genes with an absolute value of F.C.>2 and p <0.05 were selected for a group intercomparison using a Venn diagram to determine which genes were unique to the DN group. Differentially expressed genes were subjected to GO-Analysis, using the over-representation test within the PANTHER classification system (Panther version 16, pantherdb.org/)[12], to identify the gene ontology for all upregulated genes.

qPCR assays.

Fourteen genes from the DN group were selected for qPCR analyses (CCNB1, PFKL, CASP2, METTL22, ITGAM, IP6K1, ARMC6, TROAP, SUSD6, PCP4, FAM229A, CACFD1, FAM129B, and CMTM4) and HPRT as housekeeping gene control. The oligonucleotide sequences are showed in Supplementary Table 1. cDNA was synthesized from each RNA sample using the enzyme SuperScript II 200 U/µl (Invitrogen, USA) according to the manufacturer's instructions. The amplifications were carried out in L.C. 480 (Roche, USA) with "SSoFast™ EvaGreen®" (BioRad, USA) according to the manufacturer's instructions with 50 ng of cDNA. The relative expression of each gene was calculated using the equation 2-ΔΔCt [13].

Results

Identification of Differential Expressed Genes (DEGs)

Based on the cutoff criteria described in the section above, a set of 351 genes was identified as differentially expressed in the DN group versus DM-2 group, including 345 up-regulated and six down-regulated. The representative heat map of 70 distinctive genes is shown in Figure 1. CCNBI was the most up-regulated gene with an FC of 2.9428. Table 2 lists the top 14 microarray up-regulated genes selected for the qPCR assays. The gene expression profiles for each group are clustered using Euclidean and ward algorithms.

RT-PCR analyses

To verify the expression level of the up-regulated mRNAs, we performed RT-PCR for the genes described in Table 2. The RT-PCR results confirm the microarray data in only four genes (PFKL, CCBN1, CASP2, and METTL22). As shown in Figure 2, gene casp2 has an increased expression in the groups of DM-2 and DN and is differentially expressed in the

group of DN from PREDM and CTRL, in a similar manner PFKL and CCNB1 genes showed a trend to have an increased expression from PREDM to DN and are differentially expressed in the group of DN versus all groups; in the case of METTL22 the DN group had the highest relative expression.

Evaluation of CCNB1, PFKL, CASP2, and METTL22 as possible biomarkers

To evaluate the usefulness of these genes as potential biomarkers to identify patients with DN, we performed ROC curves. In Figure 3, the ROC analysis showed significant differences of the genes compared to the non-discrimination line. Furthermore, the AUC for all four genes was above 0.75, suggesting their utility as biomarkers. Surprisingly, METTL22 had the highest AUC (>.9719), sensitivity, and specificity, and therefore can serve as the most promising biomarker for DN.

Upregulated genes are involved in pathways related to DN development.

Panther database gene ontology analysis showed 42 different pathways. In Figure 4, Wnt signaling pathway was the most represented with 3 genes (Lrp6, SMARCC2, GNG13), CCNB1 gene was related to cell cycle and p53 pathways; PFKL was related to glycolysis, and CASP2 is related to intrinsic apoptotic signaling pathway in response to DNA damage (not shown in figure 4). METTL22 as being a new gene has non-associated pathways in the database.

Discussion

DN is a pathological state resulting from the dysregulation of multiple genetic interactions involved in complex metabolic pathways. Furthermore, increased oxidative stress induced by hyperglycemia may contribute to diabetic complications such as DN. In general, the study of alterations in the expression of genes associated with metabolic disorders can be beneficial in the search for biomarkers of diagnostic and prognostic utility. Currently, the validated markers to differentiate the progression of DN from PREDM2 states or in the early stages of DM2 can be performed by comparing the transcriptional expression between groups of subjects with different stages of evolution of the disease. The identification of these markers is essential to improve the clinical management of patients with DN. To date, no reliable markers have been developed related to altered gene regulation associated with abrupt changes in insulin and glucose metabolism during DN. Numerous trials have been

carried out using different omic technologies to find new biomarkers for diabetic nephropathy or to identify early signs of this disease, this with the idea of replacing the measurement of albuminuria since DN can frequently progress without an increase in albumin excretion [14].

A microarray analysis this way can identify biomarkers genes for early DN diagnosis. In this study, a set of more than 300 genes were upregulated in the group of DN compared to CTRLs, PREDM, and DM-2 groups of subjects when transcriptional RNA profiles from blood samples were analyzed using a human chip containing more than 61k DNA sequence probes (including mRNA and non-coding long RNAs). Further analysis by RT-PCR in 14 of these 300 upregulated genes confirmed the overexpression of four genes in DN patients: CASP2, PFKL, METTL22, and CCNB1 but lower expression levels in PREDM and DM-2 subjects, suggesting that these genes are critical in the evolution of DN. ROC analysis for these four genes showed values from 0.9719, 0.8853, 0.8533 and 0.7748, respectively, suggesting they can be used as potential biomarkers to diagnose DN.

From our results, CASP2 showed 4 times fold increased RNA expression associated with DN patients. Casp2 is an enzyme belonging to the caspase's family; caspases mediate cellular apoptosis through the proteolytic cleavage of specific protein substrates. The encoded protein may function in stress-induced cell death pathways and cell cycle maintenance. Although CASP2 has not been reported in other DN microarray analyses, CASP2 has been linked to apoptosis-associated to the development of DN, explicitly with mitochondrial injury, due to a positive feedback loop, caspases damage the mitochondria, leading to loss of mitochondrial transmembrane potential [15, 16]. CASP-2 activates cytochrome C and Bax translocation that, in a positive loop, causes further mitochondrial damage [17]. To confirm these results, methodologies to evaluate markers for apoptosis in blood cells to know if overexpression of CASP2 is linked to DN.

On the other hand, Ccnb1 is a cell cycle-related protein. CCNB1 codifies for Ccnb1 and has been found overexpressed in transcriptomic analyses related to podocytes injury damage [18]. However, its active role in DN has not been further explored. The function of CCNB1 is associated with mitosis, given that this protein is necessary for proper control of the G2/M transition phase of the cell cycle [19]. Woroniecka et al. found CCNB1 differentially

expressed in kidney tubule samples from DN patients [20]; Tsai et al. evaluated CCNB1 levels by RNA-seq from proximal tubular epithelial cells; they found CCNB1 to be associated with Skp2, a protein associated with cellular senescence in DN [21]. In the injured proximal tubule (PT), FoxM1 regulates podocyte cell cycle progression, and known downstream targets of FoxM1 include Ccnb1. Podocyte injury damage could be precluded by CCNB1 overexpression mediated by FOXM1, which indeed suppressed in vitro proliferation, implying FoxM1 as a PT-specific regulator of injury-induced cell proliferation, being CCNB1 involved in the loss of podocytes in the case of renal injury as it has been previously documented [22, 23]. The increase in CCNB1expression could be related to podocyte injury damage, and this could be analyzed in terms of FoxM1increase cell expression. Interestingly, FOXR2 was repressed in diabetes groups compared to controls in the microarray analysis we have done, suggesting that this gene expression also changes when the diabetes imbalance has begun. Our results indicate that CCNB1 has three times the fold overexpression in DN patients' blood cells compared to CTRLS, PREDM, and DM-2. Clinically these DN patients have short clinical evolution, with DN diagnosed on a one-year window since first clinical or laboratory manifestations. Thus, CCNB1 may be upregulated early in DN patients. Type 2 diabetes is a complex disorder with diminished insulin secretion and insulin action contributing to hyperglycemia and many metabolic defects that underlie the disease [24]. PFKL is an enzyme that catalyzes the conversion of D-fructose 6-phosphate to D-fructose 1,6-bisphosphate, which is a crucial step in glycolysis [25]. β cell glycolysis increases insulin secretion in a glucose concentration-dependent manner and could link between impaired glucose metabolism and impaired insulin secretion. Transgenic mice overexpressing the PFKL gene were associated with diminished glucose-induced insulin [26]. PFKL gene upregulation has not been reported before in another microarray; Some previous results implicate PFKL in the dysregulation of energy management, and DM-2 mediated damage in DN associated with Smad4[27]. As hyperglycemia is intrinsically linked with ROS production, shifting the glucose balance enables ROS to activate intracellular signaling and/or induce cellular damage and cell death [28]. In a correlation analysis (data not shown), we found that the higher RNA PFKL expression in the group of DN patients studied could be associated with other oxidative stress genes confirming an altered signal pathway relevant to DN evolution. METTL22 is a

newly reported gene not known, to our knowledge, linked with DN. METTL22 is a member of the non-histone lysine methyltransferases. It interacts with its substrate, Kin17, which is involved in DNA repair and replication and mRNA processing [29]. Oxidative stress plays a crucial role in the development of vascular complications of diabetes. Several reports have also shown that diabetes increases oxidative damage to DNA [30, 31]. METTL22 overexpression in DN could be explained as an activation of the DNA damage process fragmentation, which has already been used as a predictive marker for DN [32]. Other biomarkers such as urinary 8-hydroxydeoxyguanosine are associated with DNA damage and may be used to diagnose DN [33]. As DNA damage occurs, overexpression of these genes could be happening simultaneously since our RT-PCR results only show overexpression of CCNB1, METTL22, and PFKL in DN patients, whereas CASP2 gene expression increases in PREDM, DM-2, and overexpressed in DN. Integrated cohort analyses are necessary to identify these molecules as prognostic markers for DN.

Numerous assays have been made using different omics technologies to find new biomarkers or to identify early signs of disease that replace the standard diagnostic criteria that is albuminuria, as DN can frequently progress without an increase in albumin excretion [14]. Several biomarkers have been proposed before, with AUC values ranging from <0.6 to 0.9. For example, Transferrin/Creatinine Ratio (TCRE) excretion have demonstrated an AUC of >0.8 [34]. Still, as many new proposed biomarkers have special considerations, TCRE has increased concentration in a non-diabetic population with hypertension [35]. In the end, just a few biomarkers are being used in clinical trials [4]. The four proposed biomarkers in our study have good AUC values, with METTL22 having the highest AUC value (0.97), but further studies are needed to find usage considerations or prospective validation for these markers.

In this study, through transcriptome analyses, we were able to identify four blood biomarkers involved in pathways related to DN (CASP2, PFKL, METTL22, and CCNB1) using mRNA microarrays and qPCR. These gene expressions also occur in prediabetic and diabetic groups and patients with DN, suggesting they may be valuable biomarkers in early-stage diagnosis. METTL22 had a higher AUC value of 0.9719 and is worthy of further evaluation in a higher population sample. However, this study has some limitations. For example, these predictions were not validated in cohort experiments. The number of

samples used for analysis is small. Despite this, the results were statistically significant. Further studies will analyze a bigger sample, and experimental data may validate the value of the identified biomarker genes for DN.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Figure Legends

Fig. 1. Cluster analysis of genes associated with Diabetic nephropathy; A) Top 70 genes are selected in the figure and B) Cluster analysis of genes associated selected for RT-PCR analysis. Samples of total RNA from patients with DN (dark blue), controls (light pink), PREDM (light blue), and DM-2 (purple) were used to identify the transcriptional profile associated with each of the representative groups by microarray analysis (8X60k v2, Agilent, USA). Gene expression profiles for each group are clustered using Euclidean and ward algorithms with feature autoscaling. Color Scale represents green down-regulated, red over-expressed, and black genes without gene expression change.

Fig. 2. Gene expression analyses of CCNB1, PFKL, CASP2, and METTL22 in ND patients. Gene expression analysis by microarray was carried out on cDNAs from blood samples to assess selected genes' relative gene expression profile in DN. The graphs show the median as a descriptive statistic. All relative values used HPRT expression as the reference gene in equations $2^{-\Delta\Delta Ct}$. Multiple comparison tests were performed using the non-parametric Kruskal-Wallis test. P values less than 0.05 were considered statistically significant. The * represent significant differences, ** very significant differences, and *** highly significant differences.

Fig. 3. Receiver operating characteristic (ROC) curves of potential diabetic nephropathy gene biomarkers. The ROC analysis and area under the curve (AUC) are shown for CTRLS, PREDM, DM-2, and DN, for all CCNB1, PFKL, CASP2, and METTL22 RNAs. The middle dash represents the line of no discrimination. The *p* values of less than 0.05 were considered statistically significant.

Fig. 4. Pathway analysis of up-regulated genes. PANTHER GO-Slim Pathway process analysis identified 42 pathways associated with the 345 up-regulated genes from DN. Pie chart representing all the identified pathways, each pie color represents a different pathway (right). Each association is supported by literature evidence as to the pathway component extension shows.

Supplementary Fig. 1. Venn diagram depicting mRNA fingerprint per group, Gene mRNA specific to each group was selected from those differentially expressed. The overlapping numbers stand for the mutual differentially expressed genes between the

different comparisons, and the non-overlapping numbers specify the genes unique to each group. The top number represents upregulated genes, and the bottom represents down-regulated genes.

Supplementary Fig. 2. Gene expression analyses for genes vary from microarray analysis. Gene expression analysis by microarray was carried out on cDNAs from blood samples to assess selected genes' relative gene expression profile in DN. The graphs show the median as a descriptive statistic. All relative values used HPRT expression as the reference gene in equations $2^{-\Delta\Delta Ct}$. Multiple comparison tests were performed using the non-parametric Kruskal-Wallis test.

Table legends

Table 1: Sociodemographic, epidemiological, and clinical characteristics of the study participants by group.

Table 2: Top 14 up-regulated genes.

Table 3: Receiver operating characteristic curve of genes selected in DN.

Supplementary table 1. Gene primers.

TABLES

Table 1: Sociodemographic, epidemiological, and clinical characteristics of the study participants by group.

Variables	CTRLS	PREDM	DM-2	DN	P value
Age (years)	43.93	51.38	50.63	47.14	ns
M/F	4/11	4/11	5/10	4/11	ns
Hypertension (n)	5	8	6	13	0.0039
IMC	27.69 +/- 5.78	32.06 +/- 5.47	32.13 +/- 6.22	31.40 +/- 5.55	ns
GFR (ml/min/1.73 m²)	107.2+/- 17.63	105.7 +/- 14.92	108.2 +/- 15.1	81.37 +/- 35.71	0.0054*
Serum Creatinine	0.7933 +/- 0.15	0.7533 +/- 0.14	0.7467 +/- 0.15	1.12 +/- 0.49	0.0012*
Hb1Ac (%)	5.331+/-0.23	5.995+/-0.18	8.131+/-1.8	8.179+/-1.924	<0.0001
Hemoglobin (g/dl)	14.96 +/- 1.60	15.51 +/- 1.06	14.92 +/- 1.46	14.45 +/- 2.03	ns
Leucocytes ($\times 10^{3/ul}$)	6.26 +/- 1.66	7.49 +/- 1.62	7.35 +/- 1.52	7.81 +/- 1.14	0.0362[A]
Monocytes (%)	7.04 +/- 1.35	6.08 +/-1.7	6.45 +/-1.60	6.3 +/- 1.10	ns
Lymphocytes (%)	32.33 +/- 6.78	33.87 +/- 8.82	36.67 +/- 5.7	32.57 +/- 7.19	ns
Neutrophils (%)	58.13 +/- 7.2	57.08 +/- 9.02	54.34 +/- 6.46	58.29 +/- 7.63	ns

All variables are represented in mean +/- Standard Deviation, [A]= CTRLS vs DN , *= DN vs all Groups, ns= non significant.

Table 2: Top 14 up-regulated genes.

ID	GENE NAME	LogFold Change	Adjusted Value	*p*
CCNB1	Cyclin B1	2.94289068	5.35E-06	
PFKL	Phosphofructokinase	2.90571555	0.00055807	
ITGAM	Integrin subunit alpha M	2.75183391	0.00062043	
IP6K1	Inositol hexakisphosphate kinase 1	2.66290617	0.00309512	
ARMC6	Armadillo repeat containing 6	2.63551298	0.00032945	
CASP2	Caspase 2	2.58327823	0.00149083	
SUSD6	Sushi domain containing 6	2.56958755	0.0045901	
TROAP	Trophinin associated protein	2.56561544	0.00184783	
METTL22	Methyltransferase like 22	2.50223787	0.00032642	
PCP4	Purkinje cell protein 4	2.46924684	0.00048389	
FAM229A	Family with sequence similarity 229 member A	2.4567957	0.00249063	
CACFD1	Calcium channel flower domain containing 1	2.42058333	0.0005273	
FAM129B	Family with sequence similarity 129 member B	2.4166741	0.00108924	
CMTM4	CKLF like MARVEL transmembrane domain containing 4	2.37524045	0.00305938	

Table 3: Receiver operating characteristic curve of genes selected in DN.

Gene	Specificity%	Sensitivity%	Cut-off	AUC (CI)	*p* Value
CCNB1	71.11	86.67	0.9774	0.8533(0.7588-0.9479)	<0.0001
PFKL	90	73.53	0.9344	0.8853(0.7815- 0.9891)	0.0002
CASP2	51.11	80	1.233	0.7748(0.6230-0.9266)	0.0015
METTL22	86.67	100	1.523	0.9719(0.9353-1.000)	<0.0001

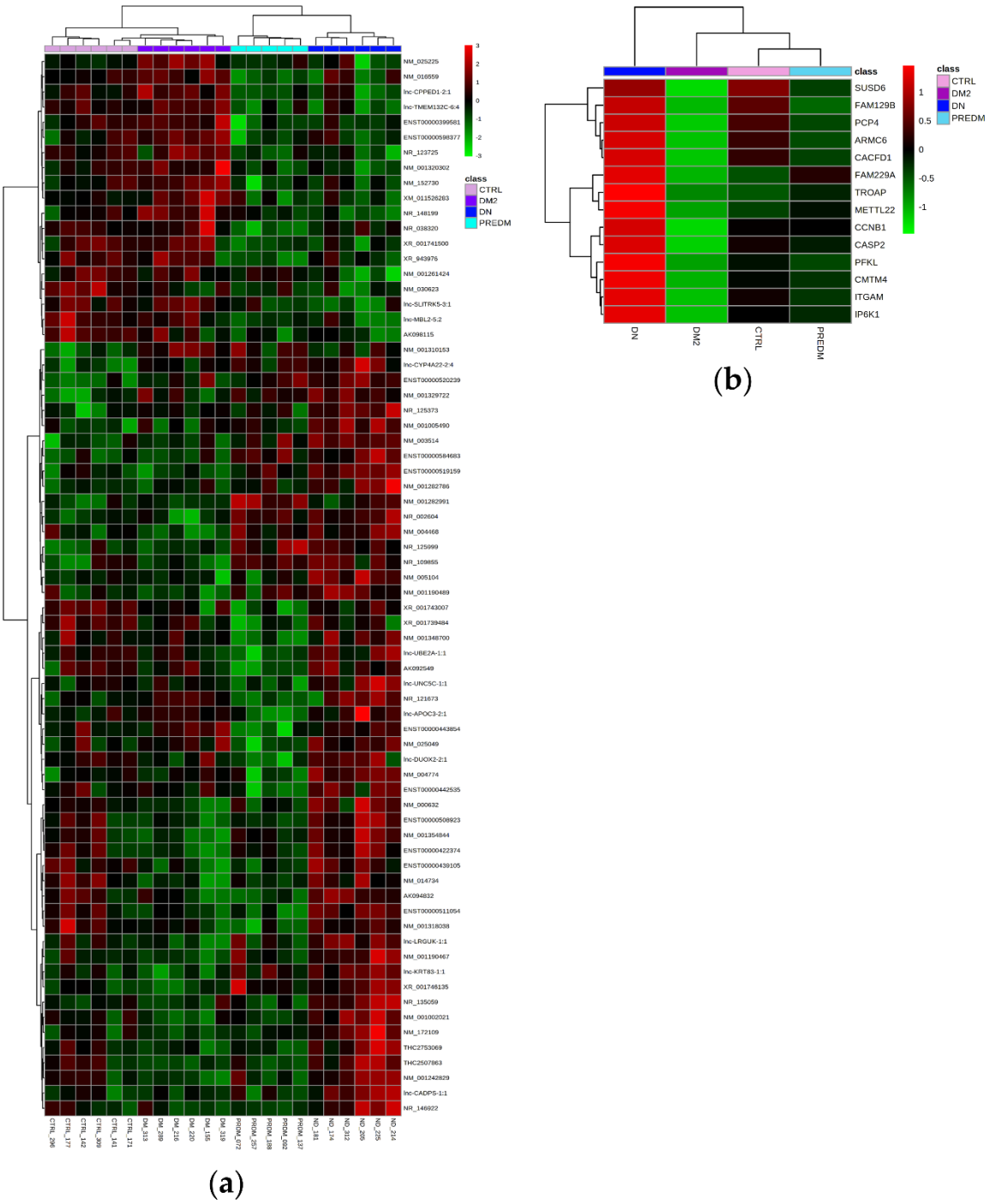
Specificity and sensitivity of Gene RNA expression determined from comparison between DN vs all groups. ROC curves were calculated using 2 -2-ΔΔCt. AUC = area under curve.

Supplementary table 1. Gene primers.

Name	Sequence
CCNB1-F	AAGGCTGTGGCAAAGGTGTA
CCNB1-R	AAGGGGCCACAAGCTTTATT
PFKL2-F	CTTCCTCATCTACGAGGGCT
PFKL2-R	GGATGATGTTGGAGACGCTCA
ITGAM-F	GGTTCACCTCCTTCCAGGTT
ITGAM-R	TTGCGTTCTCTTGGAAGGTCA
IP6K1-F	AGTTGCGTTTGCTTTGGACC
IP6K1-R	GCAGCGCTAGGTGTACAGAA
ARMC6-F	GCAGTTTGAATCGCAAGGGG
ARMC6-R	ATGTCATGTGTGGGCTCCTG

CASP2-F	TTGGGCTATGACGTCCATGT
CASP2-R	TCGGTGTGCAGGTAACTGTG
SUSD6-F	AGCAGGCAGTGTCATCGAAT
SUSD6-R	TCTCCATGGCTGGTTTCCAC
TROAP-F	GCAAGATTCCGGTACGCTCT
TROAP-R	TGGTTTCTGCACCCATCTCC
METTL22-F	ACGACGACTTGACTGATGCT
METTL22-R	TGTGACGTCCAAGTGTCTCA
PCP4-F	GGTGGCCATTCAGTCTCAGT
PCP4-R	GTGGACTAGGAGGGGGTTCT
FAM229A-F	TCGACGTCTACCTCGCCAT
FAM229A-R	CATCATTGTCTCGTGCTCGG
CACFD1-F	TCCTACCCAGGTAGTGGGAC
CACFD1-R	CTTGATCCACAGCCAACCCT
NIBAN2-F	GGGCTGGTGCTCTACGAAAA
NIBAN2-R	GTCCACGGACGTGAGGATTT
CMTM4-F	TGATCTTGGCCCTGATTGCAT
CMTM4-R	CAAGACGCCAGTCACCACAA
HPRT-F	TGACCTTGATTTATTTTGCATACC
HPRT-R	CGAGCAAGACGTTCAGTCCT

Figure 1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CTRL
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DN
PREDM
SUSD6
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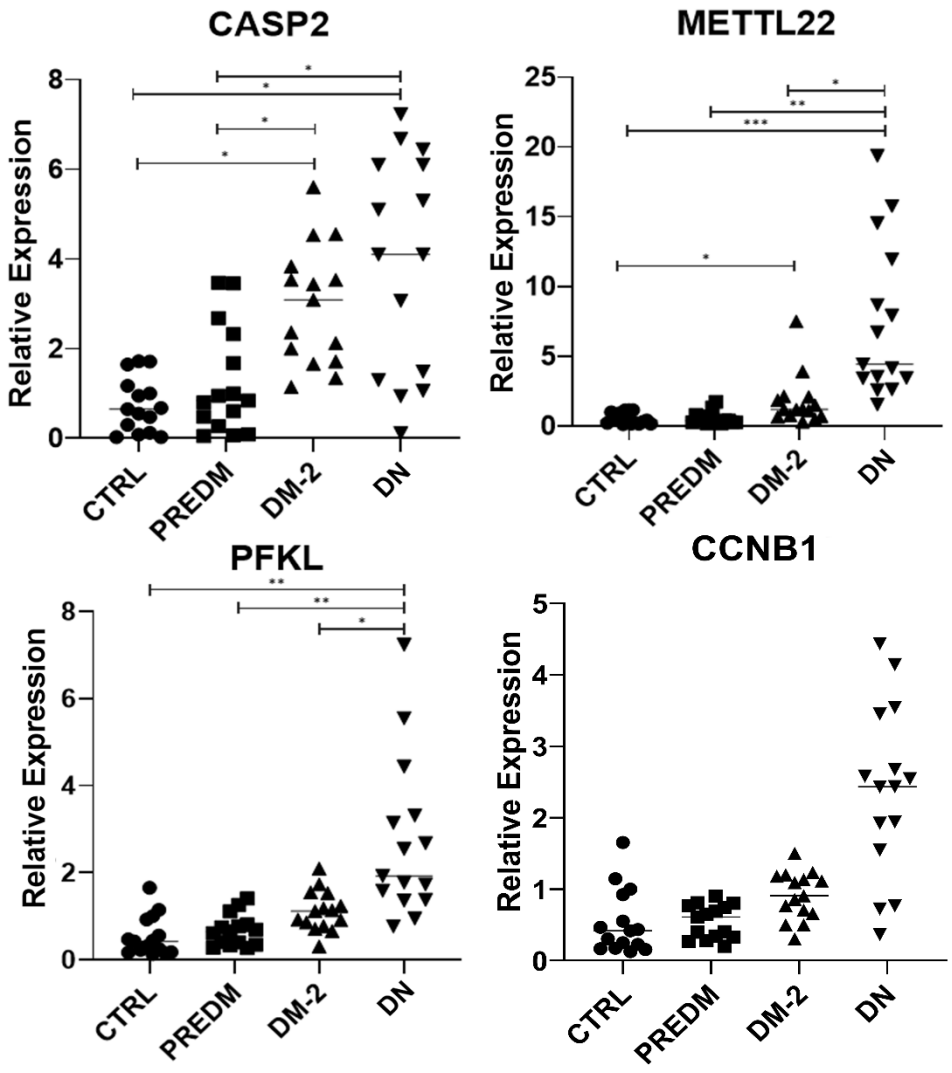
DM2
CTRL
PREDM
(b)
(a)

Figure 2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METTL22
PFKL
CCNB1
Relative Expression
CTRL
PREDM
DM-2
DN

Figure 3

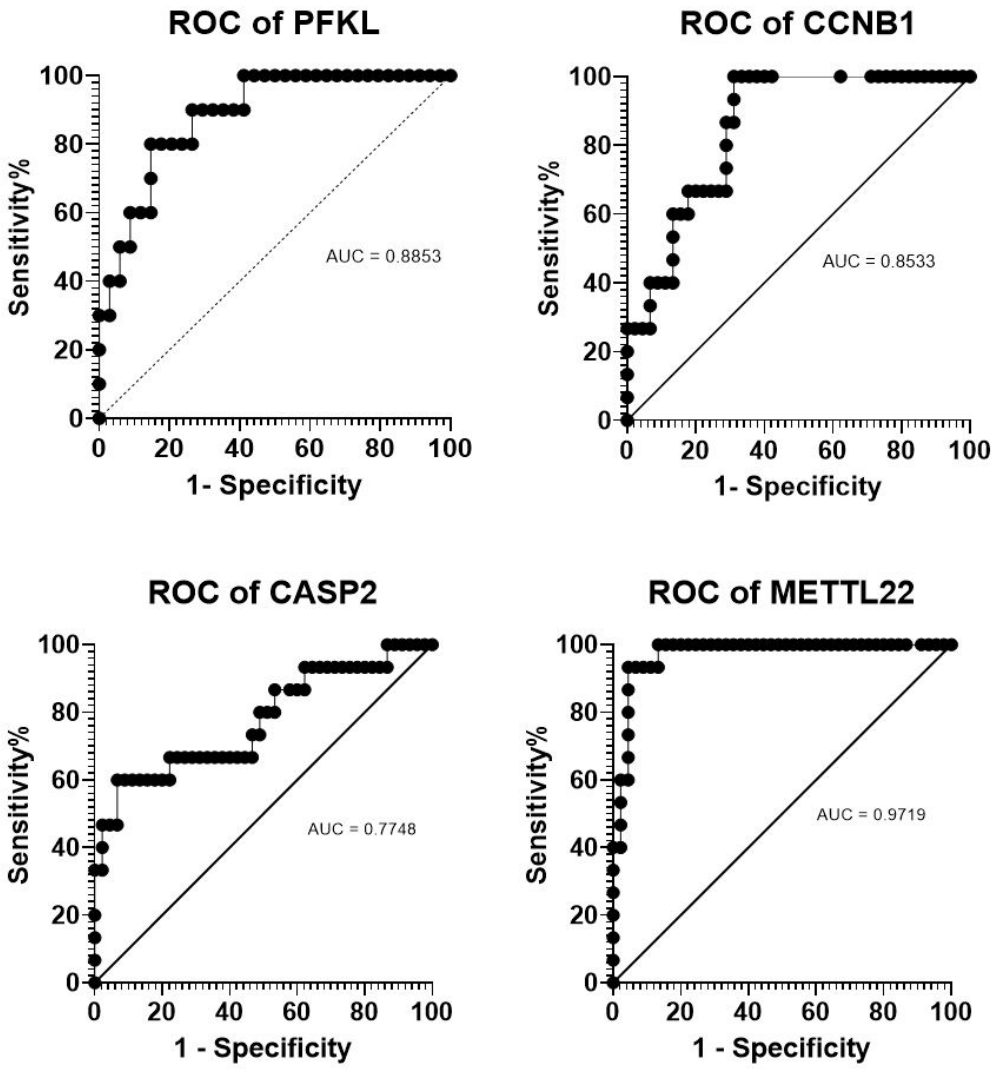
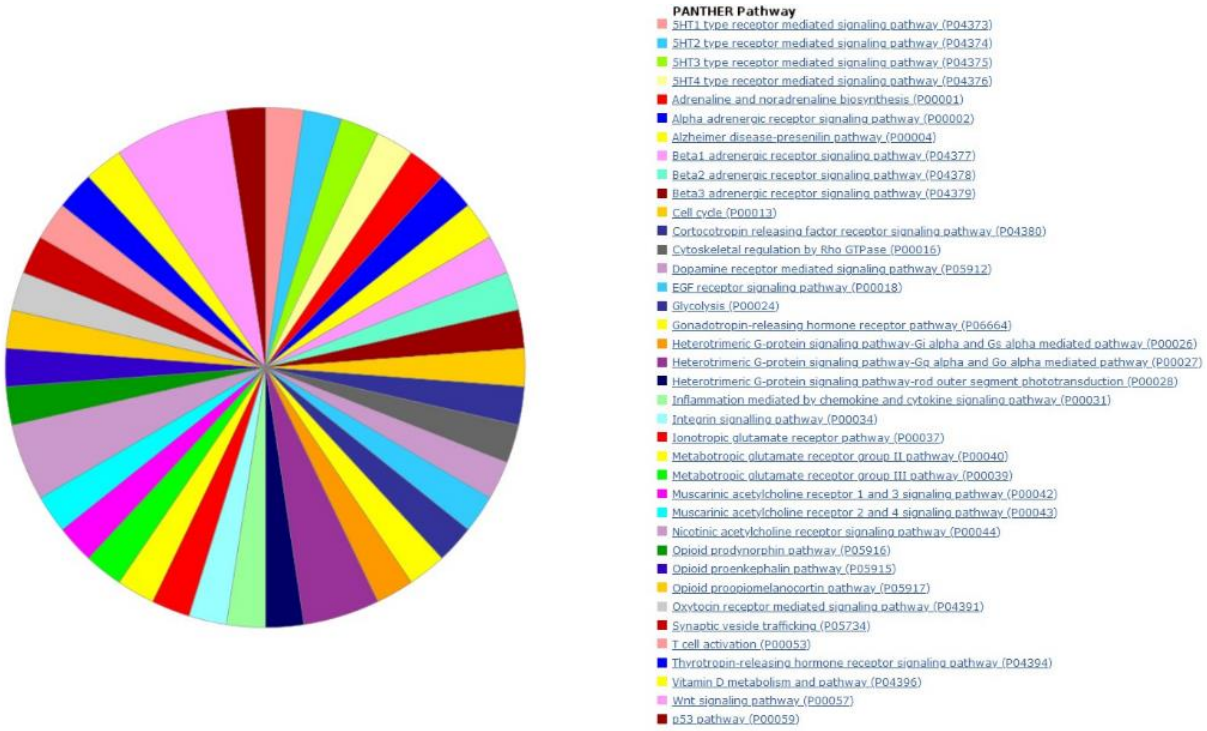

ROC of PFKL
Sensitivity%
1- Specificity
AUC = 0.8853
ROC of CCNB1
Sensitivity%
1- Specificity
AUC = 0.8533
ROC of CASP2
Sensitivity%
1 - Specificity
AUC = 0.7748
ROC of METTL22
Sensitivity%
1 - Specificity
AUC = 0.9719

Figure 4


PANTHER Pathway
5HT1 type receptor mediated signaling pathway (P04373)
5HT2 type receptor mediated signaling pathway (P04374)
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5HT4 type receptor mediated signaling pathway (P04376)
Adrenaline and noradrenaline biosynthesis (P00001)
Alpha adrenergic receptor signaling pathway (P00002)
Alzheimer disease-presenilin pathway (P00004)
Beta1 adrenergic receptor signaling pathway (P04377)
Beta2 adrenergic receptor signaling pathway (P04378)
Beta3 adrenergic receptor signaling pathway (P04379)
Cell cycle (P00013)
Cortocotropin releasing factor receptor signaling pathway (P04380)
Cytoskeletal regulation by Rho GTPase (P00016)
Dopamine receptor mediated signaling pathway (P05912)
EGF receptor signaling pathway (P00018)
Glycolysis (P00024)
Gonadotropin-releasing hormone receptor pathway (P06664)
Heterotrimeric G-protein signaling pathway-Gi alpha and Gs alpha mediated pathway (P00026)
Heterotrimeric G-protein signaling pathway-Gq alpha and Go alpha mediated pathway (P00027)
Heterotrimeric G-protein signaling pathway-rod outer segment phototransduction (P00028)
Inflammation mediated by chemokine and cytokine signaling pathway (P00031)
Integrin signalling pathway (P00034)
Ionotropic glutamate receptor pathway (P00037)
Metabotropic glutamate receptor group II pathway (P00040)
Metabotropic glutamate receptor group III pathway (P00039)
Muscarinic acetylcholine receptor 1 and 3 signaling pathway (P00042)
Muscarinic acetylcholine receptor 2 and 4 signaling pathway (P00043)
Nicotinic acetylcholine receptor signaling pathway (P00044)
Opioid prodynorphin pathway (P05916)
Opioid proenkephalin pathway (P05915)
Opioid proopiomelanocortin pathway (P05917)
Oxytocin receptor mediated signaling pathway (P04391)
Synaptic vesicle trafficking (P05734)
T cell activation (P00053)
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Figure Supplementary 1

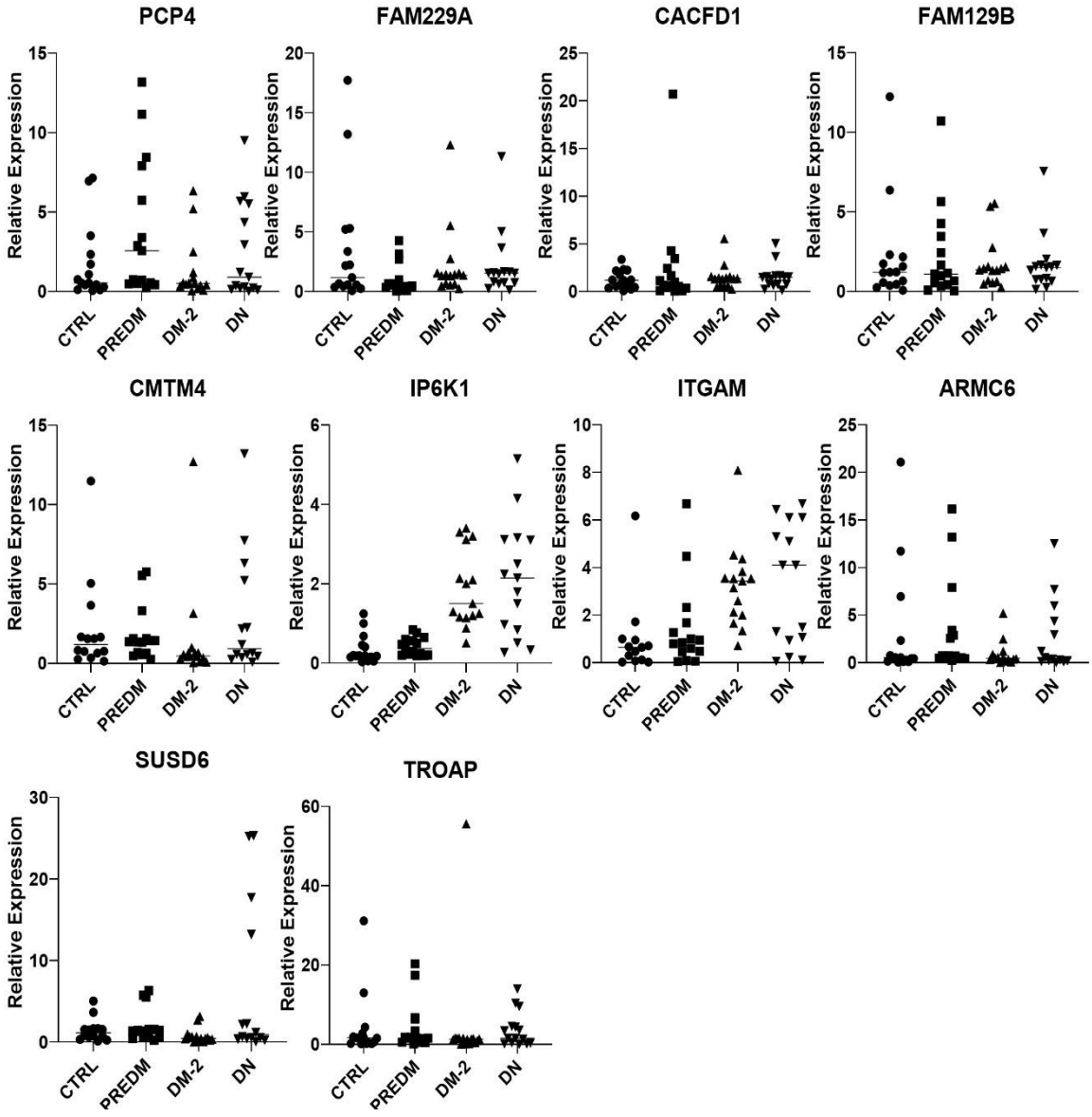
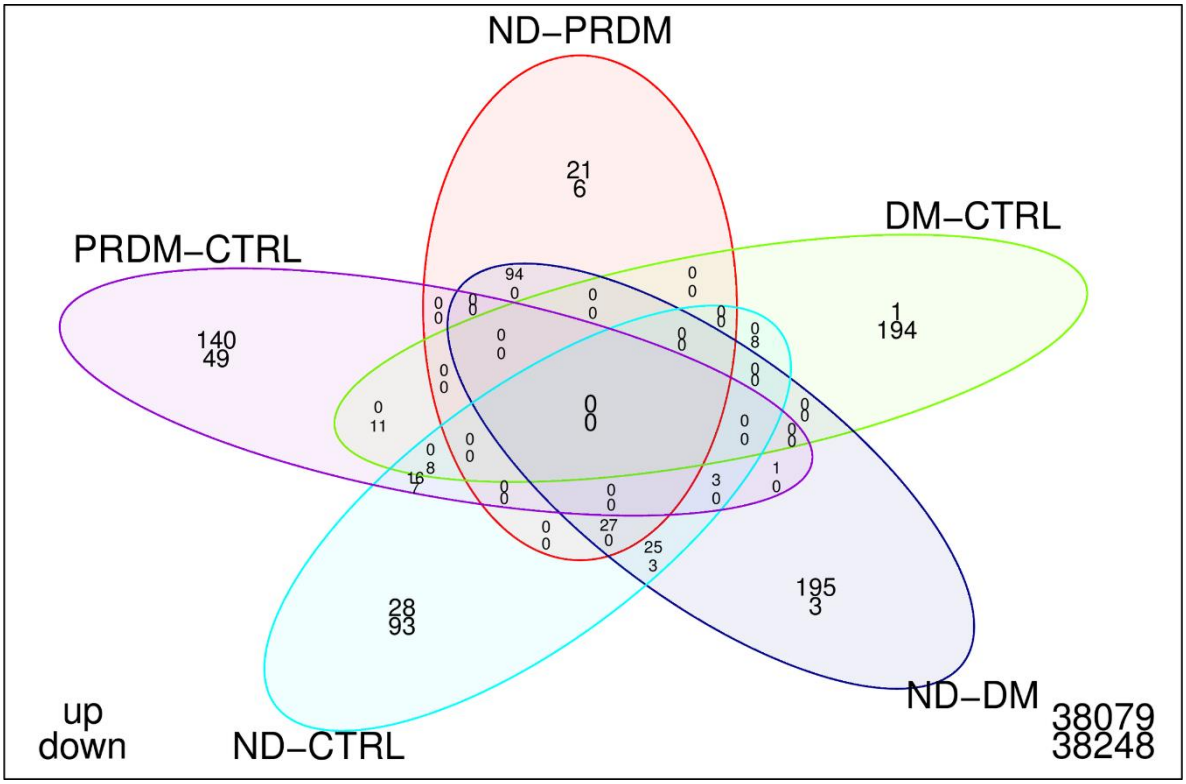


Figure supplementary 2



Archives of Medical Research

Transcriptome analysis identifies oxidative stress injury biomarkers for Diabetic Nephropathy

--Manuscript Draft--

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Abstract:	Background. The early diagnosis of diabetic nephropathy (DN) is essential to improve the prognosis and manage patients affected by this disease. Standard biomarkers, including albuminuria and glomerular filtration rate, are limited to give a precise result. New molecular biomarkers are needed to identify better and predict DN disease evolution. Characteristic DN biomarkers can be identified using transcriptomic analysis. Aim of the study. To evaluate the transcriptomic profile of controls (CTRLs, n = 15), prediabetes (PREDM, n = 15),, type-2 diabetes mellitus (DM-2, n = 15), and DN (n = 15) patients by microarray analysis to find new biomarkers, RT-PCR was used to confirm gene biomarkers specific for DN. Materials and methods. Blood samples were used to isolate RNA for microarray expression microarrays evaluating 26,803 unique gene sequences and 30,606 LncRNA sequences, selected gene biomarkers for DN were validated using qPCR assays. Sensitivity, specificity, and area under the curve (AUC) were calculated as measures of diagnostic accuracy. Results. The DN transcriptome, founding here, were composed by 300 induced genes, compared to CTRLs, PREDM, and DM-2 groups. RT-qPCR assays validated that METLL22, PFKL , CCNB1 and CASP2 genes were induced in the DN group compared to CTRLs, PREDM, and DM-2 groups. The ROC analysis for these four genes showed 0.9719, 0.8853, 0.8533 and 0.7748 AUC values respectively. Conclusion. Among induced genes in the DN group, we found that CASP2, PFKL and CCNB1 can be used as potential biomarkers to diagnose DN, where, METLL22 represents the best with an AUC=0.9719.

ANNEXES

PUBLICATIONS

López-Hernández, Y., E. E. Lara-Ramírez, M. Salgado-Bustamante, J. A. López**, J. J. Oropeza-Valdez**, E. Jaime-Sánchez, J. E. Castañeda-Delgado, M. Magaña-Aquino, M. Murgu and J. A. Enciso-Moreno (2019). "Glycerophospholipid Metabolism Alterations in Patients with Type 2 Diabetes Mellitus and Tuberculosis Comorbidity." Archives of Medical Research 50(2): 71-78.

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metabolites

MDPI

Article

The Urinary Metabolome of Healthy Newborns

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Abstract: The knowledge of normal metabolite values for neonates is key to establishing robust cut-off values to diagnose diseases, to predict the occurrence of new diseases, to monitor a neonate's metabolism, or to assess their general health status. For full term-newborns, many reference biochemical values are available for blood, serum, plasma and cerebrospinal fluid. However, there is a surprising lack of information about normal urine concentration values for a large number of important metabolites in neonates. In the present work, we used targeted tandem mass spectrometry (MS/MS)-based metabolomic assays to identify and quantify 136 metabolites of biomedical interest in the urine from 48 healthy, full-term term neonates, collected in the first 24 h of life. In addition to this experimental study, we performed a literature review (covering the past eight years and over 500 papers) to update the references values in the Human Metabolome Database/Urine Metabolome Database (HMDB/UMDB). Notably, 86 of the experimentally measured urinary metabolites are being reported in neonates/infants for the first time and another 20 metabolites are being reported in human urine for the first time ever. Sex differences were found for 15 metabolites. The literature review allowed us to identify another 78 urinary metabolites with concentration data. As a result, reference concentration values and ranges for 378 neonatal urinary metabolites are now publicly accessible via the HMDB.

Keywords: newborn; metabolites; tandem mass spectrometry; inborn errors of metabolism; reference values

scientific reports

OPEN

Targeted metabolomics identifies high performing diagnostic and prognostic biomarkers for COVID-19

Yamilé López-Hernández[1,2✉], Joel Monárrez-Espino[3✉], Ana-Sofía Herrera-van Oostdam[4], Julio Enrique Castañeda Delgado[1,5], Lun Zhang[6], Jiamin Zheng[6], Juan José Oropeza Valdez[5], Rupasri Mandal[6], Fátima de Lourdes Ochoa González[5,7], Juan Carlos Borrego Moreno[8], Flor M. Trejo-Medinilla[2,7], Jesús Adrián López[9], José Antonio Enciso Moreno[5] & David S. Wishart[6]

Research exploring the development and outcome of COVID-19 infections has led to the need to find better diagnostic and prognostic biomarkers. This cross-sectional study used targeted metabolomics to identify potential COVID-19 biomarkers that predicted the course of the illness by assessing 110 endogenous plasma metabolites from individuals admitted to a local hospital for diagnosis/treatment. Patients were classified into four groups (= 40 each) according to standard polymerase chain reaction (PCR) COVID-19 testing and disease course: PCR−/controls (i.e., non-COVID controls), PCR+/not-hospitalized, PCR+/hospitalized, and PCR+/intubated. Blood samples were collected within 2 days of admission/PCR testing. Metabolite concentration data, demographic data and clinical data were used to propose biomarkers and develop optimal regression models for the diagnosis and prognosis of COVID-19. The area under the receiver operating characteristic curve (AUC; 95% CI) was used to assess each models' predictive value. A panel that included the kynurenine: tryptophan ratio, lysoPC a C26:0, and pyruvic acid discriminated non-COVID controls from PCR+/not-hospitalized (AUC = 0.947; 95% CI 0.931–0.962). A second panel consisting of C10:2, butyric acid, and pyruvic acid distinguished PCR+/not-hospitalized from PCR+/hospitalized and PCR+/intubated (AUC = 0.975; 95% CI 0.968–0.983). Only lysoPC a C28:0 differentiated PCR+/hospitalized from PCR+/intubated patients (AUC = 0.770; 95% CI 0.736–0.803). If additional studies with targeted metabolomics confirm the diagnostic value of these plasma biomarkers, such panels could eventually be of clinical use in medical practice.

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), first identified in China in December 2019, is responsible for the coronavirus disease outbreak (COVID-19)[1]. One year after the pandemic was declared, the infection has caused nearly 2.5 million deaths worldwide[2]. In spite of significant efforts undertaken by government and health authorities to contain the spread, the virus continues to wreak havoc around the world.

Since COVID-19 can lead to multi-organ dysfunction, disease severity is not only the result of pathogen burden[3], but also the consequence of the host's immune response to the infection. It is well known that viruses hijack the host cell machinery for self-replication, as they compete for nutrients and other metabolites to satisfy their bioenergetic and biosynthetic requirements. This metabolic hijacking can lead to an alteration of the host's metabolome[4]. In fact, a number of metabolic pathways have already been found to be consistently altered (glycolysis, fatty acid synthesis, glutaminolysis, pyrimidine metabolism, and tryptophan/kynurenine metabolism) in

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PLOS ONE

RESEARCH ARTICLE

Immunometabolic signatures predict risk of progression to sepsis in COVID-19

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Abstract

Viral sepsis has been proposed as an accurate term to describe all multisystemic dysregulations and clinical findings in severe and critically ill COVID-19 patients. The adoption of this term may help the implementation of more accurate strategies of early diagnosis, prognosis, and in-hospital treatment. We accurately quantified 110 metabolites using targeted metabolomics, and 13 cytokines/chemokines in plasma samples of 121 COVID-19 patients with different levels of severity, and 37 non-COVID-19 individuals. Analyses revealed an integrated host-dependent dysregulation of inflammatory cytokines, neutrophil activation chemokines, glycolysis, mitochondrial metabolism, amino acid metabolism, polyamine synthesis, and lipid metabolism typical of sepsis processes distinctive of a mild disease. Dysregulated metabolites and cytokines/chemokines showed differential correlation patterns in mild and critically ill patients, indicating a crosstalk between metabolism and hyperinflammation. Using multivariate analysis, powerful models for diagnosis and prognosis of COVID-19 induced sepsis were generated, as well as for mortality prediction among septic patients. A metabolite panel made of kynurenine/tryptophan ratio, IL-6, LysoPC a C18:2, and phenylalanine discriminated non-COVID-19 from sepsis patients with an area under the curve (AUC (95%CI)) of 0.991 (0.986–0.995), with sensitivity of 0.978 (0.963–0.992) and specificity of 0.920 (0.890–0.949). The panel that included C10:2, IL-6, NLR, and C5 discriminated mild patients from sepsis patients with an AUC (95%CI) of 0.965 (0.952–0.977), with sensitivity of 0.993(0.984–1.000) and specificity of 0.851 (0.815–0.887). The panel with citric acid, LysoPC a C28:1, neutrophil-lymphocyte ratio (NLR) and kynurenine/tryptophan ratio discriminated severe patients from sepsis patients with an AUC (95%CI) of 0.829

metabolites

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Article

Urinary Metabolomic Profile of Neonates Born to Women with Gestational Diabetes Mellitus

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Abstract: Gestational diabetes mellitus (GDM) is one of the most frequent pregnancy complications with potential adverse outcomes for mothers and newborns. Its effects on the newborn appear during the neonatal period or early childhood. Therefore, an early diagnosis is crucial to prevent the development of chronic diseases later in adult life. In this study, the urinary metabolome of babies born to GDM mothers was characterized. In total, 144 neonatal and maternal (second and third trimesters of pregnancy) urinary samples were analyzed using targeted metabolomics, combining liquid chromatographic mass spectrometry (LC-MS/MS) and flow injection analysis mass spectrometry (FIA-MS/MS) techniques. We provide here the neonatal urinary concentration values of 101 metabolites for 26 newborns born to GDM mothers and 22 newborns born to healthy mothers. The univariate analysis of these metabolites revealed statistical differences in 11 metabolites. Multivariate analyses revealed a differential metabolic profile in newborns of GDM mothers characterized by dysregulation of acylcarnitines, amino acids, and polyamine metabolism. Levels of hexadecenoylcarnitine (C16:1) and spermine were also higher in newborns of GDM mothers. The maternal urinary metabolome revealed significant differences in butyric, isobutyric, and uric acid in the second and third trimesters of pregnancy. These metabolic alterations point to the impact of GDM in the neonatal period.

Keywords: newborns; metabolomics; gestational diabetes; pregnancy

1. Introduction

Gestational diabetes mellitus (GDM), defined as hyperglycemia during gestation [1], is the most frequent medical complication in pregnancy, affecting 7–10% of all pregnancies worldwide [2]. GDM has been associated with various adverse outcomes both in mothers and newborns. Women with GDM are at higher risk of developing type 2 diabetes (T2D), mainly 3–6 years after delivery, and their offspring is also at higher risk of becoming overweight and obese [3]. The metabolic abnormalities associated with GDM include increased

diagnostics

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Article

Kynurenine and Hemoglobin as Sex-Specific Variables in COVID-19 Patients: A Machine Learning and Genetic Algorithms Approach

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Abstract: Differences in clinical manifestations, immune response, metabolic alterations, and outcomes (including disease severity and mortality) between men and women with COVID-19 have been reported since the pandemic outbreak, making it necessary to implement sex-specific biomarkers for disease diagnosis and treatment. This study aimed to identify sex-associated differences in COVID-19 patients by means of a genetic algorithm (GALGO) and machine learning, employing support vector machine (SVM) and logistic regression (LR) for the data analysis. Both algorithms identified kynurenine and hemoglobin as the most important variables to distinguish between men and women with COVID-19. LR and SVM identified C10:1, cough, and lysoPC a 14:0 to discriminate between men with COVID-19 from men without, with LR being the best model. In the case of women with COVID-19 vs. women without, SVM had a higher performance, and both models identified a higher number of variables, including 10:2, lysoPC a C26:0, lysoPC a C28:0, alpha-ketoglutaric acid, lactic acid, cough, fever, anosmia, and dysgeusia. Our results demonstrate that differences in sexes have implications in the diagnosis and outcome of the disease. Further, genetic and machine learning algorithms are useful tools to predict sex-associated differences in COVID-19.

Keywords: COVID-19; sex; machine learning; metabolomics

1. Introduction

Sex differences in manifestations of viral infections have been observed for multiple respiratory viruses [1,2] where men have shown higher disease severity and mortality compared with women, including SARS-CoV [3], MERS-CoV [4], the H1N1 pandemic [5],

Universidad Autónoma de San Luis Potosí
Posgrado en Ciencias Biomédicas Básicas

DEFENSA DOCTORAL

"Análisis metabolómico y transcriptómico diferencial de pacientes prediabéticos, diabéticos y con nefropatía diabética para identificar potenciales biomarcadores de daño renal"

PARA OBTENER EL GRADO DE:
DOCTOR EN CIENCIAS BIOMÉDICAS BÁSICAS

Presenta:
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1

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Epidemiología de la DM

- Prevalencia mundial aproximada del 8.8 al 10.5% en adultos de 20 a 79 años
- México reporto un aumento del 14% (2000 al 2021) al 16.9% , en el 2017 el INEGI atribuyo a la DM el 14% de las defunciones totales del país.

537 millones de adultos viven con diabetes

3 de 4 adultos con diabetes viven en país medios/bajos recursos

6.7 millones de muertes en el 2021

International Diabetes Federation. *IDF Diabetes Atlas*, 10th edn. Brussels, Belgium.
INEGI "estadísticas a propósito del día mundial de diabetes. 14 Nov 2021"

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Complicaciones

- Se estima que los pacientes diabéticos tendrán 1 o más complicaciones a lo largo de su vida si no manejan un control glucémico adecuado.
  - Enfermedad periodontal
  - Pie diabético
  - Retinopatía Diabética
  - ECV
  - **Nefropatía Diabética 28% (ERC o TRR)**
- La TRR (60% asociadas a DM-2) en el 2009 fue el 3er lugar en gasto por padecimientos en el IMSS (4,712 millones).

International Diabetes Federation. *IDF Diabetes Atlas*, 8th. Brussels, Belgium, Midete Asumiendo el Control de la Diabetes, Fundación Asumiendo el Control

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Biomarcadores actuales para ND

Biomarcador	Sensibilidad	Especificidad	ABC
RBP/CRE	0.547	0.600	0.572
IL-18	0.609	0.606	0.691
Cistatina C	0.630	0.526	0.588
Resistina	0.691	0.546	0.642
NGAL/CRE	[illegible]	[illegible]	0.601
YKL-40	0.386	[illegible]	0.455
TNF-α	0.776	0.407	0.602
IL-6	0.457	0.651	0.560
VCAM1	0.555	0.518	0.517
Adiponectina	[illegible]	[illegible]	0.511
IL-1α	0.518	0.597	0.538
E-selectin	[illegible]	[illegible]	0.501
CRP	[illegible]	[illegible]	[illegible]
ICAM1	0.534	[illegible]	0.504
Leptina	0.477	0.594	0.552
MCP-1	[illegible]	[illegible]	[illegible]
P-selectina	0.598	0.460	0.504
L-selectina	[illegible]	[illegible]	[illegible]
PAI-1	0.491	0.385	0.399
Fetuina A	[illegible]	0.705	0.283

- Albuminuria >30mg/día

Etapas Intermedias de la enfermedad

Oscilante

Nefropatía diabética No Proteinúrica

- TFG (Creatinina)

Normalmente identificada cuando existe una disminución significativa <60 ml/min

NUEVOS BIOMARCADORES DESDE ETAPAS TEMPRANAS DE LA ND SON NECESARIOS

- Biomarcadores Candidatos

Cystatin C sérica | Pigment epithelium derived factor (PEDF) | KIM1 (Kidney Injury Marker 1) | Laminin | NGAL | TGF-β1 | MCP-1

Jorge L et al. Diabetic Nephropathy: Diagnosis, Prevention, and Treatment *Diabetes Care* 2005

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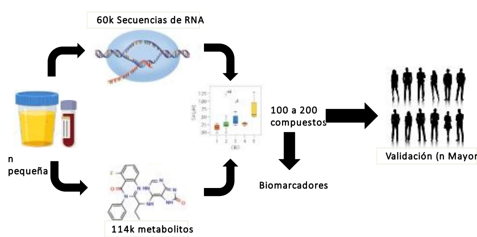
Herramientas ómicas



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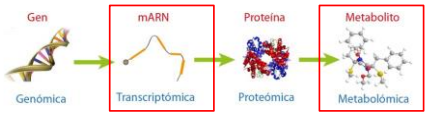
Huella Omica en Nefropatía Diabética

- Estudiar en conjunto la huella transcripcional y el metaboloma de la enfermedad nos podría permitir identificar
  - Biomarcadores de daño renal



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Metabolómica en Nefropatía Diabética

Autor	Método	Muestra	Etapa	Metabolitos encontrados	
Suhre y cols.	GC-MS	Suero	3 y 4	Glicerofosfolipidos, ácidos grasos Libres	⇧
Zhao y cols.	HPLC	Suero	4	Metabolismo de ácidos grasos libres y los intermediarios del ciclo de Krebs	⇧
Connor y cols.	H-NMR	Tejido de Riñón (Ratones)	Modelo	Metabolismo de aminoácidos y el ciclo de Urea	⇩

Weisset al.Metabolomicsin KidneyDiseases. 2011.NatureNephrologyReviews

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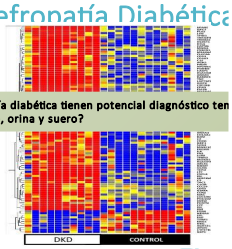
Transcriptómica en Nefropatía Diabética

Woroniecka y cols. Identificó la Huella transcripcional en microdisecciones de riñón en pacientes con ND

¿Los metabolitos o genes que se presentan en nefropatía diabética tienen potencial diagnóstico ten se presentan en sangre, orina y suero?

Increase in DKD	1273	653	347	115
Decrease in DKD	558	100	39	13



Woronieckaet al. Transcriptome analysis of human diabetic kidney disease. 2011. Diabetes

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Justificación

- La DM es un problema de salud mundial que va en aumento
- Los pacientes con DM presentan complicaciones como la ND.
- No existen biomarcadores tempranos confiables para ND o de daño renal temprano
- No se han publicado estudios de transcriptómica y metabolómica en población mexicana con nefropatía diabética
- Este estudio sentaría el precedente en explorar esto

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Hipótesis

Existe huella transcriptómica en sangre y metabolómica en suero y orina asociado a daño renal temprano y/o nefropatía diabética en pacientes con DM-2.

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Objetivo General

Determinar y analizar el metaboloma y el transcriptoma en sujetos sanos, con prediabetes, diabetes mellitus tipo 2 y con nefropatía diabética con el fin de identificar potenciales biomarcadores de daño renal temprano.

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Objetivos Específicos

1. Obtener el perfil metabólomico y transcriptómico de sujetos con prediabetes, diabetes mellitus tipo 2, DM2 con nefropatía diabética y controles sanos.
2. Identificar los metabolitos y genes diferencialmente expresados asociados a nefropatía diabética.
3. Identificar los metabolitos y genes diferencialmente expresados que puedan utilizarse como potenciales biomarcadores de daño renal temprano.

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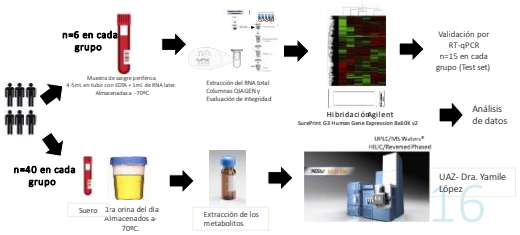
Metodología General



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Selección de la población de estudio

Grupos	Criterios de inclusión	Criterios de exclusión
Sujetos Sanos	• Edad 30 a 75 años • Filtración glomerular > 90 y < 125 ml/min/1.73m2 estimada con la ecuación CKD-EPI • Sin hipertensión • Sin albuminuria o proteinuria	• Sujetos con infecciones urinarias • Infecciones por virus de Hepatitis o VIH • Mujeres embarazadas • Enfermedades autoinmunes • Mujeres en periodo menstrual • Que no firme el consentimiento informado
Prediabetes	• Edad 30 a 75 años • Glucemia en ayunas 100 -110 mg/dl • HbA1c ≥ 5.7 % y < 6.4% • Filtración glomerular > 90 y < 125 ml/min/1.73m2 estimada con la ecuación CKD-EPI, sin albuminuria	**Factores a considerar** • Arsénico en orina > 20 ug/g creat • Flúor en orina > 1.5 ppm
Diabetes Mellitus Tipo 2	• Edad 30 a 75 años • Glucemia en ayunas ≥ 126 mg/dl • HbA1c ≥ 6.5% • Filtración glomerular > 90 y < 125 ml/min/1.73m2 estimada con la ecuación CKD-EPI, sin albuminuria • Diabetes establecida < 5 años de evolución.	
Nefropatía Diabética	• Edad 30 a 75 años • Diabetes mayor a 5 años de evolución • HbA1c ≥ 6.5% • Nefropatía diabética confirmada mediante microalbuminuria (30 a 300 mg/24hr) o macroalbuminuria (> 300 mg/24hr) • Filtración glomerular < 90 a 15 ml/min/1.73m2 o > 140 ml/min/1.73m2	

Todos los pacientes serán reclutados de las Ciudades Guadalupe y Zacatecas/Zac.

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Análisis de microarreglos

SurePrint G3 Human Gene Expression 8x60k v3 Microarray (Todas las secuencias de mRNA + LncRNAs)

Dr. Edgar Lara Ramírez

Resultados comparaciones

	PRDM CTRL	PRDM DM	CTRL NEDM	NDCTRL	ND PRDM
Down	75	4 221	6 119	6	
Up	160	59	1 345	99	142

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Sujetos de estudio

Tabla 1 Características generales de los sujetos de estudio

	CTRLs	PRE-DIABETES	DM-2	ND	Valor de p (<0.05)
N total (160)	40	40	40	40	
Edad (Años) ±D.E.	47.98±12.62	52.2±11.64	52.55±8.728	53.45±11.38	ns
Sexo (M:F)	18:22	17:23	18:22	19:¥21	ns
HbA1c (%) ±D.E	5.368±0.24	6.059±0.31	8.435±2.01	7.53±1.52	<0.0001
IMC ±D.E.	27.37±4.827	31.87±5.14	31.18±5.6	31.02±5.73	0.0028 (vs CTRLs)
TFG (ml/min/1.73m2)	111.7±9.6	106.0±14.07	107.83±14.97	66.34±34.421	0.0013

Se han reclutado hasta el momento 345 sujetos.
- Infecciones urinarias
- Nefropatía prediabética
- Enfermedades autoinmunes

Base de datos con 90 variables sociodemográficas, clínicas y de laboratorio

D.E= Desviación Estándar, GFR= Tasa de Filtración Glomerular

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Análisis de microarreglos

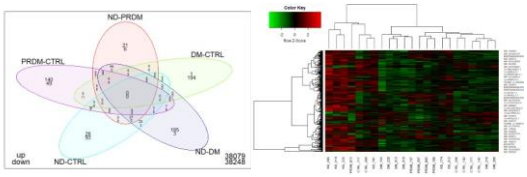

Figura 1. Diagrama Venny mapa de calor que muestra los genes con expresiones diferenciales de los resultados del microarreglo de expresión de RNA diagrama de Venn muestra 5 comparaciones. ND=nefropatía diabética, DM= Diabetes Mellitus tipo 2, PRDM= Prediabetes, CTRL= control sano.

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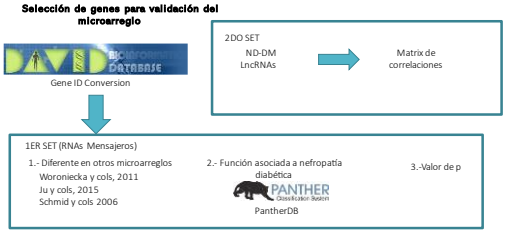
Análisis de microarreglos



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Análisis de microarreglos

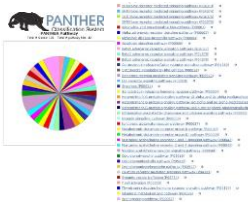

Figura3. Vías de señalización involucradas con los genes regulados a la alta resultantes de la comparación DM-DN mediante PANTHER.

Tabla4. Lista final de genes seleccionados para la validación del microarreglo

ID	Descripción
CCNB1	Cyclin B1 (CCNB1), transcriptvariant 2, mRNA
PFKL	Phosphofructokinase
ITGAM	Integrinsubunit alpha M (ITGAM)
IP6K1	Inositol hexakisphosphatekinase 1 (IP6K1)
ARMC6	Armadillo repeat containing 6(ARMC6)
CASP2	Caspase2 (CASP2)
SUSD6	Sushi domaincontaining6(SUSD6)
TROAP	Trophinin associatedprotein(TROAP)
METTL22	Methyltransferaselike 22(METTL22)
PCP4	Purkinje cell protein 4(PCP4)
FAM229A	Family with sequence similarity 229 member A(FAM229A)
CACFD1	Calcium channel flower domain containing 1(CACFD1)
FAM129B	Family with sequence similarity 129 member B(FAM129B)
CMTM4	CKLF like MARVEL transmembrane domain containing 4(CMTM4)

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Resultados Validación Microarreglos

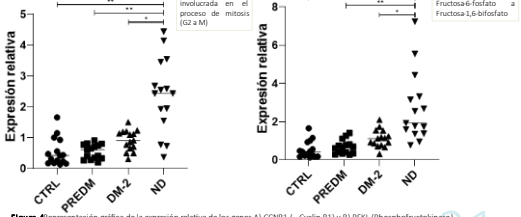

Figura 4. Representación gráfica de la expresión relativa de los genes A) CCNB1 (Cyclin B1) y B) PFKL (Phosphofructokinase). Expresión relativa determinada con el método de $2^{-\Delta\Delta CT}$, valor de p significativo <0.05, n=15 por grupo. CTRL (Controles), PREDM (Prediabetes), DM -2 (Diabetes Mellitus Tipo 2) y ND (Nefropatía Diabética).

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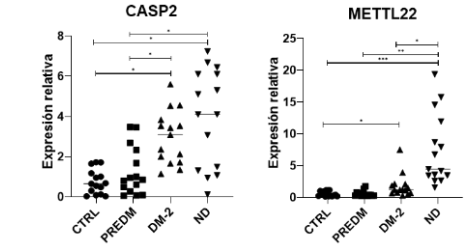

Figura4. Representación gráfica de la expresión relativa de los genes A) Caspase 2(CASP2) y B) Methyltransferase like 22(METTL22). Expresión relativa determinada con el método de $2^{-\Delta\Delta CT}$, valor de p significativo <0.05, n=15 por grupo. CTRL (Controles), PREDM (Prediabetes), DM-2 (Diabetes Mellitus Tipo 2) y ND (Nefropatía Diabética).

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Resultados Validación Microarreglos

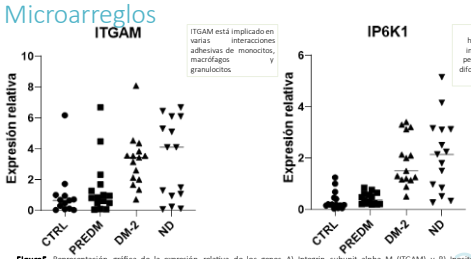

Figura5. Representación gráfica de la expresión relativa de los genes A) Integrin subunit alpha M (ITGAM) y B) Inositol hexakisphosphate kinase 1(IP6K1). Expresión relativa determinada con el método de $2^{-\Delta\Delta CT}$, valor de p significativo <0.05, n=15 por grupo. CTRL (Controles), PREDM (Prediabetes), DM-2 (Diabetes Mellitus Tipo 2) y ND (Nefropatía Diabética).

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Capacidad Diagnóstica

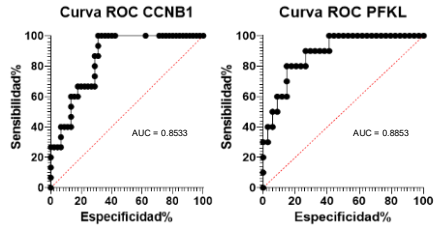

Figura 6. Curva ROC con los datos de expresión relativa de A) CCNB1 (Cyclin B1) y B) PFKL (Phosphofructokinase). AUC = Area under curve.

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Análisis metabolómico de suero

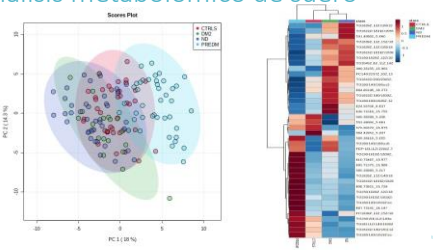

Figura 1. Análisis de componentes principales de los metabolitos en suero presentes en cada grupo. CTRLS=controles, PREDM= Prediabetes, DM2= Diabetes Mellitus tipo 2, ND= Nefropatía Diabética.

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Análisis metabolómico de orina

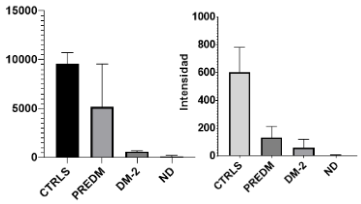

Figura 9. Intensidad de Symmetridymethilarginine. Lado izquierdo primer análisis, lado derecho 2do análisis. CTRLS=controles, PREDM= Prediabetes, T2D= Diabetes Mellitus tipo 2, T2DN= Nefropatía Diabética

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Análisis metabolómico de suero

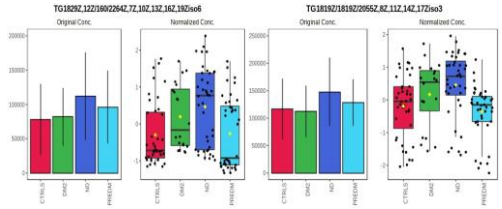

Figura 7. Metabolitos asociados a nefropatía diabética en suero. Los datos son representados en concentración relativa (valor reportado por el equipo) de lazo izquierdo y derecho es la concentración normalizada

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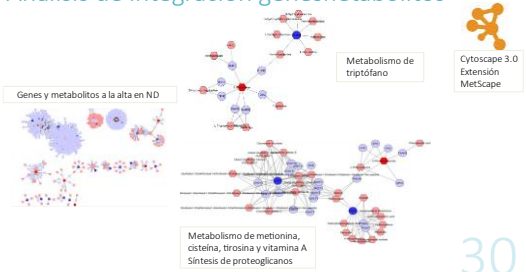
Análisis de integración genes-metabolitos



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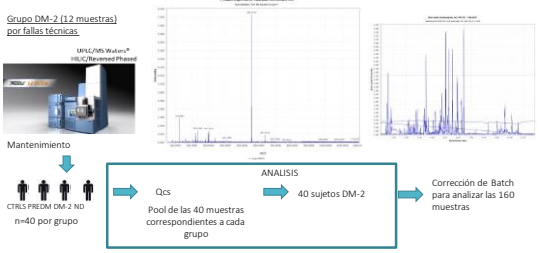
Análisis metabolómico de orina



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Comunicación científica

- 2019 . 8th Symposium of the Mexican Proteomics Society, Acapulco, Guerrero.
- 2019 . 53° Congreso Nacional de Nefrología, Zacatecas, Zac. *1er Lugar Presentación Oral*
- 2019 XXV Foro Norte de Investigación en Salud 2019 . Puerto Vallarta, Jalisco.
- 2018 . XXIII Congress of the Mexican Society of Immunology – SMI". Cancun, Quintana Roo.
- 2018 XXVII Foro nacional de Investigación en Salud. Zacatecas, Zac.

Cursos

- 2021 Curso impartido por el laboratorio de Metabolómica y Proteómica de la Universidad Autónoma de Zacatecas, **"Análisis de datos de metabolómica no dirigida"**
- 2021 Curso impartido por Scidata **"TALLER INTRODUCCION A LA CIENCIA DE DATOS CON RMARKDOWN"**
- 2019 Curso impartido por la Universidad Autónoma de Zacatecas **"The international theoretical and practical Course "Metabolomics applied to the study of human and plant diseases"**.
- 2019 Curso impartido por la UNAM **"IUIS-ALAI-SMI IMMUNOINFORMATICS COURSE".**

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Publicaciones

Archives of Medical Research

Transcriptome analysis identifies oxidative stress injury biomarkers for Diabetic Nephropathy

--Manuscript Draft--

Manuscript Number:	ARCMED-D-22-00086
Article Type:	Full Length Article
Section/Category:	Biomedical
Keywords:	diabetic nephropathy; Diabetes; transcriptome; microarray; Biomarkers
Corresponding Author:	Jose Antonio Enciso-Moreno, PhD IMSS: Instituto Mexicano del Seguro Social MEXICO
First Author:	Juan Jose Oropeza Valdez, Ph.D
Order of Authors:	Juan Jose Oropeza Valdez, Ph.D Jose de la Cruz Moreira Hernandez, MD Elena Jaime Sanchez, PhD Juan Ernesto Lopez Ramos, PhD Edgar E Lara-Ramirez, MD,PhD Yamile Lopez Hernandez, PhD Julio Enrique Castañeda-Delgado, PhD Jose Antonio Enciso Moreno, PhD
Abstract:	Background: The early diagnosis of diabetic nephropathy (DN) is essential to improve the prognosis and manage patients affected by this disease. Standard biomarkers, including albuminuria and glomerular filtration rate, are limited to give a precise result.

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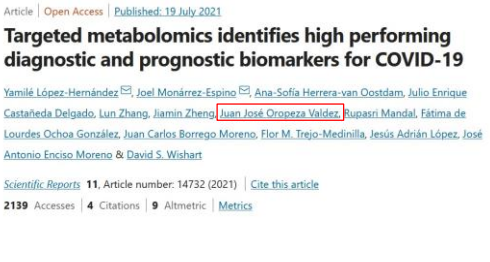

Article | Open Access | Published: 19 July 2021

Targeted metabolomics identifies high performing diagnostic and prognostic biomarkers for COVID-19

Yamilé López-Hernández, Joel Monárrez-Espino, Ana-Sofía Herrera-van Oostdam, Julio Enrique Castañeda Delgado, Lun Zhang, Jiamin Zheng, Juan José Oropeza Valdez, Rupasri Mandal, Fátima de Lourdes Ochoa González, Juan Carlos Borrego Moreno, Flor M. Trejo-Medinilla, Jesús Adrián López, José Antonio Enciso Moreno & David S. Wishart

Scientific Reports **11**, Article number: 14732 (2021) | Cite this article

2139 Accesses | **4** Citations | **9** Altmetric | Metrics

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Archives of Medical Research

Volume 50, Issue 2, February 2019, Pages 71-78

PRELIMINARY REPORT

Glycerophospholipid Metabolism Alterations in Patients with Type 2 Diabetes Mellitus and Tuberculosis Comorbidity

Yamile López-Hernández, Edgar E. Lara-Ramírez, Mariana Salgado-Bustamante, Jesús Adrián López, Juan J. Oropeza-Valdez, Elena Jaime-Sánchez, Julio E. Castañeda-Delgado, Martín Magaña-Aquino, Michael Murguı́a, José A. Enciso-Moreno

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RESEARCH ARTICLE

Immunometabolic signatures predict risk of progression to sepsis in COVID-19

Ana Sofía Herrera-Van Oostdam, Julio E. Castañeda-Delgado, Juan José Oropeza-Valdez, Juan Carlos Borrego, Joel Monárrez-Espino, Jiamin Zheng, Rupasri Mandal, Lun Zhang, Elizabeth Soto-Guzmán, Julio César Fernández-Ruiz, Fátima Ochoa-González, Flor M. Trejo Medinilla, Jesús Adrián López, [...].Yamilé López-Hernández [view all]

Published: August 30, 2021 • https://doi.org/10.1371/journal.pone.0256784

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Article

The Urinary Metabolome of Healthy Newborns

Yamilé López-Hernández [1,*], Juan José Oropeza-Valdez [2], Jorge O. Blanco-Sandate [3], Ana Sofía Herrera-Van Oostdam [4], Jiamin Zheng [5], An Chi Guo [5], Victoria Lima-Rogel [6], Rahmatollah Rajabzadeh [5], Mariana Salgado-Bustamante [4], Jesus Adrian-Lopez [7], C. G. Castillo [3], Emilia Robles Arguelles [7], Joel Monárrez-Espino [8], Rupasri Mandal [5] and David S. Wishart [5,*]

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Open Access | Article

Urinary Metabolomic Profile of Neonates Born to Women with Gestational Diabetes Mellitus

by Ana Sofía Herrera-Van Oostdam [1], Mariana Salgado-Bustamante [2], Victoria Lima-Rogel [3], Juan José Oropeza-Valdez [4], Jesús Adrián López [5], Iván Daniel Román Rodríguez [6], Juan Carlos Soto-Ortiz [7], David Alejandro Herrera-Van Oostdam [1], Yamilé López-Hernández [8,*] and Joel Monárrez-Espino [8,*]

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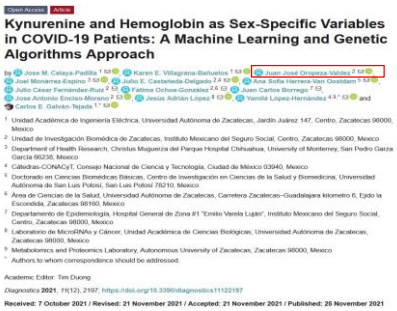

Kynurenine and Hemoglobin as Sex-Specific Variables in COVID-19 Patients: A Machine Learning and Genetic Algorithms Approach

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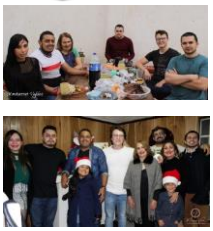
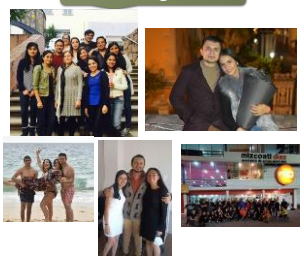
Familia



Amigos



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