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ANÁLISIS MOLECULAR Y FUNCIONAL DE LA RESPUESTA DE MACRÓFAGOS DE PACIENTES CON DIABETES A LA INFECCIÓN *in vitro* POR *Mycobacterium tuberculosis*

TESIS QUE PRESENTA

M. EN C. ELENA JAIME SÁNCHEZ

PARA OBTENER EL GRADO DE
DOCTORA EN CIENCIAS BIOMÉDICAS BÁSICAS

CODIRECTORES DE TESIS
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Potential molecular patterns for tuberculosis susceptibility in diabetic patients with poor glycaemic control: A pilot study

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Abstract

The molecular mechanisms that lead to tuberculosis-diabetes comorbidity are only partially known. In this work, a transcriptomic study focused on tuberculosis-diabetes disease associated with poor glycaemic control was carried out in patients with type 2 diabetes (DM2), as these subjects are at high risk of becoming ill with tuberculosis. Human blood samples from five groups of individuals: healthy controls (CTRL), tuberculosis (TB), TB-Type 2 diabetes comorbidity (TB-DM2), DM2 patients (HbA1c < 8.9%) and DM2 patients with poor glycaemic control (PDM2; HbA1c > 10%) were analyzed using differential expression microarrays. The differentially expressed genes (DEG) specific for TB-DM2 and PDM2 ($P < 0.05$, fold change > 2) were analyzed throughout a network strategy for the identification of potential molecular patterns linking PDM2 and TB-DM2. *OSM*, *PRKCD* and *SOCS3* were found as potential key regulatory genes of immune pathways that drives the susceptibility of PDM2 patients to develop TB. RT-qPCR assays confirmed the induction of the *OSM*, *PRKCD* and *SOCS3* genes in patients with TB-DM2. Furthermore, these molecules showed a protein-protein interaction network scored composed of 19 proteins and 30 pathways interactions. These analyzes suggest that poorly controlled DM2 leads to a transcriptional change that modifies the expression of key regulatory molecules associated with the poor immune response observed in patients with TB and provides essential information to better understand the molecular pathology of TB-DM2.

Keywords: Poor glycaemic control, diabetes-tuberculosis comorbidity, transcriptomics, molecular patterns, regulatory genes.

Introduction

People with type 2 diabetes (DM2) have higher susceptibility to develop tuberculosis (TB), and their risk increases with higher blood glucose concentrations (Lee et al. 2016). This risk has been reported by epidemiological associations and has been evaluated in prospective studies, reporting that DM2 patients with poor glycaemic control (PDM2), mainly those with values of glycated hemoglobin (HbA1c) greater than 10%, have a relative risk of developing TB 3.76 times higher compared to healthy subjects (Critchley et al. 2018). However, the molecular mechanisms that lead to the development of comorbidity between TB and DM2 (TB-DM2) in patients with PDM2 are poorly understood. Immune impairment in DM2 patients without TB has been studied in several kind of cells, showing numerous mechanisms implicated such as of metabolic pathways, ROS production, mitochondrial alterations, stress of the endoplasmic reticulum, formation of advanced glycation end products, alterations in cytokine production, etc. (Ochoa-González et al. 2020). However, no studies had focused on the study of modified cellular pathways during TB-DM2 in patients with PDM2. Furthermore, clinical evidence indicates that patients with TB-DM2 with poor glycaemic control have higher rates of therapeutic failure in anti-TB drug treatment (Lin et al. 2020), more severe clinical symptoms and higher mortality rates (Gil-Santana et al. 2016; Barreda et al. 2020), compared to patients with TB who have no DM2. All of this hinders the proper management of each individual disease in patients with TB-DM2 comorbidity.

Transcriptome analysis has provided information on TB disease that propose molecular markers of progression (from latent infection to active disease), for the diagnosis of active TB, and for the understanding of TB physiopathology (Lee et al. 2016; Zak et al. 2017). Furthermore, common differentially expressed genes (DEG) and their integrated protein networks have been found in patients with TB from different regions of the world (Blankley et al. 2016) and, therefore, have been proposed as part of the molecular mechanisms of the immune response during TB infection (Alam et al. 2019). However, for the comorbidity of TB-DM2, few transcriptomes have been performed (Prada-Medina et al. 2017; Eckold et al. 2020), none of them particularly analyzed patients with PDM2 (HbA1c > 10%), the group with the worst prognosis. The present study aimed to identify genes and their protein-protein interactome that could modulate the molecular mechanisms that lead to the development of TB in a susceptible patient with PDM2, using predictions from the transcriptome analysis of patients with PDM2 and TB-DM2.

Material and Methods

Study population. Seventy-eight subjects from Zacatecas, Durango, Veracruz, Nuevo León, and Oaxaca states of the Mexican Republic were recruited from November 2015 to December 2019. They were classified in Control (CTRL), DM2 (HbA1c <8.9%), PDM2 (HbA1c> 10%), TB and TB-DM2 comorbidity groups. DM2, PDM2 and TB-DM2 patients were diagnosed according to the American Diabetes Association (ADA). Each new case of tuberculosis was confirmed throughout smear or GeneExpert® and culture of *Mycobacterium tuberculosis* (*Mtb*). PPD (purified protein derivative, Sanofi Pasteur, France) was administered with an intradermal injection to subjects in the CTRL, DM2, and PDM2 groups according to the US Food and Drug Administration and manufacturer's instructions, as well as QuantiFERON-TB Gold Plus (QFT, Qiagen, USA). Subjects in non-TB groups were included only if they had a negative result on the PPD or QFT tests. Individuals with HIV infection, PPD application eight weeks before sample obtention, TB patients with anti-tuberculosis treatment for more than fifteen days (Bloom et al. 2012), and individuals taking immunosuppressive drugs were not included in the study.

The National Committee for Scientific Research and Ethics of the Instituto Mexicano del Seguro Social (IMSS), approved the study (R-2013-785-001 and R-2018-785-118) according to the international ethical regulations of the Helsinki Convention for research studies in humans (World Medical Association, 2001). All participants signed an informed consent letter.

Biological samples. Whole blood samples were obtained from each participant in vacutainer-EDTA tubes (two aliquots of five ml per subject) and then mixed with RNAlater (Thermo Fisher Scientific, USA) in a 5:1 proportion and stored at -70°C until use. The PPD application to qualified individuals was performed after the collection of the blood sample.

Sample RNA isolation and labeling. The Trizol-Chloroform method (Invitrogen, USA) coupled to the QIAmp column protocol (Qiagen Inc, USA) was used for processing frozen blood samples. RNA concentration and purity were determined using ND-1000 nanodrop (Thermo Fisher Scientific, USA). The RNA integrity number (RIN) was determined using the RNA 6000 Nano kit (Agilent Technologies, USA), on a Bioanalyzer-2100 equipment (Agilent Technologies Genomics, USA) following the manufacturer's instructions. Only RNA samples with 260/280> 2 and RIN> 6 were used for microarray

assays. The synthesis of complementary Cy3-labeled copy RNA (cRNA) was done according to the standard low input Quick Amp labeling protocol (Agilent Technologies, USA), using 200 ng of total RNA.

Expression microarray assays. Transcriptional analysis profiles were performed after hybridization of the Cy3-cRNA samples with high-density human GE 4X44K v2 microarrays (Part Number: G4845A, Agilent Technologies, USA) for 17 h at 65°C, according to the manufacturer´s instructions. The mean fluorescence intensity (MFI) values of each probe on the chip were obtained using the SureScan Microarray Scanner laser reader (G4900DA, Agilent Technologies, USA) and validated using the Agilent Feature Extraction program (Agilent Technologies, USA).

Microarray statistical analysis. Statistical processing of the microarray raw data was performed within the R (RRID:SCR_001905) environment (R Foundation for Statistical Computing, 2016) applying the functions from Bioconductor and Limma R-packages. The raw data were corrected with the 'norm exp' function. The 'quantile' function was applied to normalize and correct the batch effect among the arrays. The 'aver exp' function was applied to produce the average between the arrays, which was used as input to fit the linear regression models, considering the comparisons at the disease level. Then, the empirical Bayes function (eBayes), which was applied to the resulting linear contrast models, allowed the determination of induced and repressed genes, based on the fold change values extracted from the 'decide test' function (Smyth 2004). This function used a p value < 0.05 and a logFC value of one. Intergroup comparisons (IntGC) with CTRL were performed for the DM2 and PDM2 groups (IntGC1) and for the DM2, PDM2, TB, and TB-DM2 groups (IntGC2). The gene aver exp of the significant values ($p<0.05$) in the IntGC2 was represented on a heat map. The identification of DEG for each IntGC was made through the construction of Venn (RRID:SCR_016561) diagrams (Oliveros 2015). Subsequent analyses were made focused on PDM2 (from IntGC1) and TB-DM2 (from IntGC2) specific DEG.

Prediction of key regulatory genes for PDM2 and TB-DM2 and combining them. Specific DEG lists for both groups (PDM2 and TB-DM2) were uploaded to the "Enrichr" (RRID:SCR_001575) platform (Tan et al. 2013; Kuleshov et al. 2016). DEG signaling pathways of PDM2 and TB-DM2 were identified and merged from four Enrichr databases (Keeg, Reactome, WikiPathways and BioCarta 2016). Subsequent analysis was made with the molecular patterns of both PDM2 and TB-DM2 DEG. From these results, we identified similar signaling pathway terms between databases, considering the combined scores provided

by Enrichr (Tan et al. 2013; Kuleshov et al. 2016). These scores were averaged to rank potential key regulatory genes (KRG). For example, similar terms from the Reactome, BioCarta and WikiPathways databases (Interleukin-6 family signaling, IL 6 signaling pathway and IL-6 signaling pathway, respectively) were obtained and considered as the consensus term 'IL-6 family signaling'. The combined score average of 16.97* were calculated with the above terms and the genes were submitted from every original term as follows: 'SOCS3, OSM, IL6R and PRKCD'. In addition, network analysis was performed using the Cytoscape (RRID:SCR_005748) program version 3.5.1 (Schwikowski et al. 2003), and some KRG were predicted for each case (PDM2, TB-DM2, and the combination of both PDM2 and TB-DM2 groups).

RT-qPCR for OSM, PRKCD, and SOCS3. First, 2.5 μg of total RNA from 78 samples were converted to cDNA using the Superscript II enzyme (Invitrogen, USA), following the manufacturer's instructions. *OSM*, *PRKCD*, and *SOCS3* were selected for RT-qPCR validation of the microarray and *HPRT* for basal normalizations. Sequences of oligonucleotides are shown in Supplementary Table 2. RT-qPCR amplifications were performed with 50 ng of cDNA from each sample using a LightCycler 480 thermocycler (Roche, USA) with SSoFast EvaGreen® master mix (BioRad, USA) according to the manufacturer's instructions. The relative expression of each gene was calculated by the $2^{-\Delta\Delta Ct}$ equation (Livak and Schmittgen 2001). Comparisons of relative expression between groups were made with the Kruskal Wallis test and Dunn post hoc, with a confidence interval of 95% in the GraphPad Prism (RRID:SCR_002798) 6.0 program (GraphPad Software, San Diego, California, USA).

Protein-Protein Interaction Analysis

Finally, the protein-protein interaction network for OSM, PRKCD and SOCS3 was produced with inBio Discover™ using a relevance score cutoff of 1 (Li et al. 2017). Then, these results were also analyzed on the STRING (RRID:SCR_005223) platform using the highest confidence value of 0.9 and a false discovery rate (FDR) of 1 (Snel et al., 2000; Szklarczyk et al., 2021).

Results

Study population

The clinical characteristics of the microarray (n = 20) and RT-qPCR (n = 78) populations are shown in Supplementary Table 1. Age and sex had significant differences for RT-qPCR ($p < 0.0001$ and 0.0226,

respectively). Glucose and HbA1c also had notable differences between individuals (microarray: $p = 0.0082$ and 0.0077, respectively; RT-qPCR: both $p < 0.0001$).

Poor glycaemic control and tuberculosis modulate the transcriptional profile of patients with diabetes

To inquire about the transcriptional profile of PDM2 patients, we performed an intergroup comparison with CTRL for the DM2 and PDM2 groups (IntGC1) (Fig. 1a and 1b). A unique differential transcriptional profile was found for the PDM2 group, composed of seventy-one induced genes and ninety-nine repressed genes (Figs. 1a and 1b). Then, to clarify the transcriptional profiling of TB-DM2, we performed another intergroup comparison with CTRL for the DM2, PDM2, TB and TB-DM2 groups (IntGC2) (Figs. 1c and 1d). The transcriptional profile of TB-DM2 and TB shared twenty induced and one hundred repressed genes. The transcriptional profile of TB-DM2 showed a set of thirty-six induced and forty-eight repressed specific DEG (Figs. 1c and 1d). TB-DM2 and PDM2, shared two repressed pseudogenes (RPL7P44 and KRTAP5-14P). The transcriptional profile of PDM2 from the IntGC2 was composed of sixty-one induced genes and seventy-five repressed genes with respect to the TB-DM2, TB, DM2, and CTRL groups. From IntGC2, DM2 had only one induced DEG and two repressed DEG (Figs. 1c and 1d). Using the expression levels of 446 DEG ($p<0.05$) in IntGC2 (Supplementary Table 3), we built a heat map to observe the transcriptional profile of the TB-DM2, TB, PDM2, DM2 and CTRL groups (Fig. 2). A first cluster with similar transcriptional profile composed of the DM2 and CTRL groups was observed. A second cluster with the most similar transcriptional profile was also identified for the TB-DM2 and TB groups. However, the transcriptional profile of PDM2 was different between the study groups (Figs. 1c, 1d and Fig. 2).

Differential molecular patterns of PDM2 and TB-DM2 are associated with metabolism, antigen processing, infections, immune response, and cytokine expression

In search of molecular patterns of TB susceptibility in PDM2 patients, specific DEGs induced in PDM2 (from IntGC1) and TB-DM2 (from IntGC2) and registered in the HUGO Gene Nomenclature Committee (HGNC) were used for the enrichment of signaling pathways. The ECSIT, STK11, PLK3, CDC34, and KLC3 induced genes in PDM2 (Table 2), had the highest number of hits with signaling pathways such as metabolism, activity of TP53, antigenic presentation, immune system regulation, and infections by bacteria and viruses. Furthermore, we identified signaling pathways such as TNF, OSM, IL-6, IFN-γ,

adipogenesis, insulin, etc., all associated with genes induced in the TB-DM2 group. The OSM, PRKCD, SOCS3, IL-6R, MMP9, and CREB5 genes had the highest number of hits with observed signaling pathways. The molecular patterns observed for PDM2 and TB-DM2 are critical in the host response against Mtb. Is there an association between the molecular patterns of PDM2 and TB-DM2?

The molecular patterns of PDM2 and TB-DM2 predict key regulatory genes.

To find an association between the molecular patterns of PDM2 and TB-DM2, their specific DEGs were combined to perform enrichment analyzes, and their visualization was performed by networks (Supplementary Fig. 1). The prediction of KRG was made for those who had several connections with the critical PDM2 and TB-DM2 signaling pathways for the TB response, considering them as potential molecular patterns for tuberculosis susceptibility in PDM2 patients. The induced KRGs were associated with several signaling pathways such as nicotinate and nicotinamide metabolism, PI3K-Akt, adipocytokine, type C lectin receptor, innate immune system, tuberculosis infection, antigen processing, autophagy, adaptive immune system, etc. (Supplementary Fig. 1a), all essential for the TB host response. *CREB5*, *GP9*, *STK11*, *OSM*, *RGS10*, *OXER1*, *IL-6R*, *PRKCD*, *ECSIT*, *PLK3*, *CR1*, *AXL*, *SOCS3*, *CDC34*, *KLC3*, *MRC2*, *DTX3L*, *ARPC5*, *RILP*, *MMP9*, *ANK1*, and *KCNQ2* were considered induced KRGs because they had the highest number of connections to signaling pathways induced in the combination PDM2 / TB-DM2. Of these genes, *PRKCD* and *STK11* stand out for having the highest number of signaling pathways associated, 14 and 9, respectively.

Some of the signaling pathways related to repressed genes in the PDM2/TB-DM2 analysis (Supplementary Fig. 1b) included seleno amino acid metabolism, protein translation, complement activation, signal transduction, and the innate immune system. The genes with the highest number of signaling pathway interactions include *EPRS*, *CD19*, *SIGLEC15*, *SFTPA2*, *TRPC6*, *EVL*, *BIRC3*, *STAT6*, *C1S*, and *HLA-DOA*, and thus were considered as KRG involved in the signaling pathways repressed for the PDM2/TB-DM2 combination (Supplementary Fig. 1b). Of these genes, *RPS19*, *HLA-DOA*, and *STAT6* stand out as having the highest number of signaling pathways, 34, 8 and 9, respectively.

OSM, PRKCD and SOCS3 are potential key regulatory genes induced in TB-DM2 associated with PDM2

OSM, *PRKCD,* and *SOCS3* appeared redundant in the signaling pathways observed critical to the immune

response against TB, such as autophagy and the innate immune system. In Fig. 3 there is a network analysis resume taken from Supplementary Fig. 1 for these key regulatory genes. *OSM*, *PRKCD,* and *SOCS3* were linked to some genes induced in PDM2 (orange diamonds in Fig. 3) through its merged signaling pathways. Therefore, *OSM*, *PRKCD* and *SOCS3* were selected for RT-qPCR validation (their microarray results are described in Supplementary Table 4). The results of the RT-qPCR assays demonstrated that *OSM*, *PRKCD* and *SOCS3* are genes induced by TB-DM2 comorbidity compared to their expression in the DM2 or PDM2 groups ($P<0.05$ or 0.01) (Figs. 4a, b, and c).

To detect a reliable physical association coming from scored experimental evidence between *OSM*, *PRKCD*, and *SOCS3*, we enrich them with a protein-protein interaction analysis. The obtained interactome was composed of nineteen proteins and thirty interactions (Supplementary Fig. 2). The interactions of OSM, PRKCD and SOCS3 with IL6ST had a strong correlation with the experimental data, because their confidence scores were one in all cases, while the confidence scores of PRKCD and SOCS3 in the interaction with STAT3 were one and 0.28, respectively (Supplementary Table 5). These data were confirmed using the STRING platform, which showed these interactions experimentally determined in humans (Fig. 5) with a PPI enrichment p value of 1.14e-07.

Discussion

Despite the widely described immune impairment of DM2 at the organ, cellular and molecular level, the physiopathology of TB-DM2 comorbidity remains poorly understood, mainly regarding the molecular interactions implied. Therefore, we sought genes involved in the immune response against Mtb infection during TB-DM2 and the presence of common signatures in PDM2. Functional enrichment analysis revealed DEGs that were functionally enriched in type I interferon signaling pathway, innate immune response, inflammatory response, and infectious diseases, in agreement with previous reports (Eckold et al., 2020; Chuanyou et al. 2021).

Here, we describe a transcriptome prediction signature that validates the key regulatory genes OSM, PRKCD, and SOCS3, as differentially induced genes during the TB-DM2 comorbidity. While we did not find common genes regulated in PDM2 and TB-DM2, the novel analysis strategy we used showed that transcriptional changes in some regulatory genes over induced during the TB-DM2 physiopathology are potentially linked with genes already overexpressed in PDM2 patients. The consensus of enrichment analysis between four databases allowed us to identify signaling pathways within the PDM2

transcriptome potentially associated with OSM, PRKCD, and SOCS3 (Table 1 and Fig. 3).

Furthermore, validation by RT-qPCR showed that our methodology worked to identify the most reliable KRG induced in TB-DM2 comorbidity (Fig. 4) and analysis of its protein-protein interaction (Fig. 5) showed their association with experimentally determined data in humans.

As we showed, a recent report from the TANDEM consortium found with RNAseq that in patients with intermediate hyperglycemia (below the current HbA1c limit for the diagnosis of DM2), a distinctive transcriptomic change occurs with overexpression of some particular genes such as SOCS3, OSM, and MMP9 in peripheral blood cells from patients with TB (Eckold et al. 2020). These data imply that the genes regulated from intermediate hyperglycemia and during TB-DM2 comorbidity belong to altered pathways in PDM2 subjects. It mainly involves KRG associated with the high susceptibility of subjects with DM2 to develop TB, which is exacerbated in PDM2 subjects.

OSM is a protein-coding gene for the Oncostatin M cytokine. It is expressed by cell populations such as macrophages, monocytes, T cells, and dendritic cells for the immune response during TB disease (Lu et al., 2020). Increased OSM secretion by monocytes and macrophages infected with Mtb synergized with TNF-α to promote MMP-1/3 secretion by human lung fibroblasts (Cecilia M O Kane, Paul T Elkington 2008). These authors proposed OSM as a cofactor for extracellular matrix breakdown in TB and as a therapeutic target to minimize TB-associated tissue damage. OSM overexpression has been reported in highly inflamed diabetes-impaired wounds, contributing to wound inflammation under normal and impaired healing conditions (Goren et al. 2006). The effect of OSM overexpression in TB-DM2 comorbidity has not been studied.

A potential role in disease has been attributed to the biological activity of OSM (Richards 2013); and several studies have identified that OSM signaling pathways could be activated through the atypical activity of PKCδ, codified by the PRKCD gene (Smyth et al. 2006, 2015). Based on our results, we propose that overexpression of the OSM and PRKCD genes in the TB-DM2 group has a functional relationship, where PKCδ could modulate the effect of OSM. This regulation makes sense because PKCδ is a serine/threonine kinase with a contrasting apoptotic function (Duquesnes et al., 2011), which is essential for the immune response in TB. Furthermore, in an animal model of diabetes, PKCδ was involved in the progression of diabetes through glucagon induction in α-islets of pancreatic cell islets (Yamamoto et al. 2017).

Regarding the physiopathology of TB, there is a study describing that PKCδ has a contrasting role as a marker of inflammation in human TB and as an essential molecule for the optimal functioning of macrophages in mice, where the presence or absence of PKCδ defined their killing effector functions against Mtb (Parihar et al. 2018). Moreover, PKCδ levels were increased in granulomas that contained multidrug resistant strains of Mtb in humans compared to non-virulent Mtb strains. This elevation of PKCδ was associated with a suboptimal elimination of Mtb in a late infection phase (Parihar et al. 2018).

The functional role of PRKCD in the physiopathology of TB-DM2 could be associated with multiple intracellular phosphorylation targets of PKCδ. A target of PKCδ is STAT3 (Li et al. 1999), a transcriptional factor for SOCS3 (Rottenberg and Carow 2014), one of the genes induced in our TB-DM2 group. Both STAT3 and SOCS3 have been studied in the function of lymphocytes during TB (Harling et al. 2019). However, the implication of SOCS3 in the context of TB-DM2 is unknown. The SOCS3 protein is a signaling feedback-negative regulator of nearly 30 cytokines and acts by occluding the cytokine-receptor union site for JAKs proteins and ubiquitinating the activated JAKs for their subsequent proteasomal degradation. Both ways are downstream of the JAK/STAT cytokine signaling pathway (Gao et al., 2018). Transactivation of the promotor target gene prevents subsequent STAT phosphorylation, dimerization, and translocation to the nucleus (Gao et al. 2018). IL-6, IL-10, and IFN-γ are some of the cytokines regulated by SOCS3, and OSM has been reported to induce SOCS3 transcription at a level that extended beyond IL-6 in murine embryonal fibroblasts and HepG2 hepatoma cells (Stross et al. 2006). These cytokines are critical in the immune response face-to-face to Mtb infection, and SOCS3 expression in both myeloid and lymphoid cells has been reported essential for resistance against Mtb through discrete mechanisms (Carow et al. 2013). For the association with DM2, SOCS3 may cause insulin resistance as it is an insulin signaling inhibitor that blocks insulin receptor substrate 1 (IRS1), avoiding its autophosphorylation and marking IRS1 for its proteasomal degradation (Emanuelli et al. 2000).

According to our signaling pathways enrichment analysis, the confluence of OSM, PRKCD, and SOCS3 for the host's immune response against Mtb is notorious. We propose OSM, PRKCD, and SOCS3 could be operating as a team, with vital functions on the interface of the immune and metabolic systems during the physiopathology of TB-DM2. OSM could affect cells from patients with TB-DM2 comorbidity due to the cooperation of PRKCD and SOCS3, being a trigger, an intermediate, and an executor, respectively. This interaction was supported by the enrichment of the protein-protein interaction presented here. Therefore, the induction of OSM, PRKCD and SOCS3 observed in our TB-DM2 group could represent a

pathological imbalance in their activation, and interaction that could promote the unfavorable clinical outcomes presented by patients with TB-DM2 compared to those who only have TB and needs to be clarified further. On the other hand, the link between patients with PDM2 and up-regulation of OSM, PRKCD, and SOCS3 was predicted in our analysis through networks that share signaling pathways. Some examples of the predicted link between the TB-DM2 and PDM2 DEG signaling pathways. Every signaling pathway is connected with OSM, PRKCD, and SOCS3. PI3K-Akt, autophagy, and adipocytokine signaling pathways were associated with STK11. The MHC class I-mediated antigen processing and presentation signaling pathway is associated with MRC2. The adaptive immune system signaling pathway was associated with KLC3. Therefore, we proposed that STK11, KLC3, and MRC2 could be promising candidates for key regulatory genes of susceptibility to TB in patients with PDM2, linked to OSM, PRKCD, and SOCS3. However, it is necessary to validate its induction in a test set of patients with PDM2 and the performance of additional functional assays to discover its role in TB infection and the establishment of active disease in a poorly controlled glycaemic environment.

Our study showed similarities with previous transcriptomic studies published on TB-DM2 patients (Prada-Medina et al. ,2017; Eckold et al., 2020), showing similar results even though we studied a smaller population. However, a typical glycerophospholipid profile has been reported using lipidomic analysis comparing TB-DM2 and TB serum (López-Hernández et al., 2019). Therefore, previous reports and the present work point to a solid transcriptional and metabolic response during TB-DM2 comorbidity driven primarily by the infectious process but with susceptibility genes already induced in PDM2.

A limitation of this study is that the expression levels of the OSM, PRKCD and SOCS3 genes were measured in whole blood cells. Therefore, we cannot determine which cell types are the source of such transcripts. To get this information is necessary to perform further functional assays with specific cell types. Although the levels of the codified proteins OSM, PRKCD, and SOCS3 were not measured, we established a reliable protein-protein interaction network based on experimental evidence from the literature. Therefore, more studies are needed to validate the molecular interaction of each key regulatory gene and to demonstrate the functional role they play during Mtb infection in susceptible patients with PDM2. The elucidation of molecular mechanisms of interaction between PDM2 and TB could help establish therapeutic targets to face the clinical challenge that TB-DM2 represents for public health and the fight against TB propagation.

Conclusion

PRKCD, SOCS3, and OSM were validated as potential key regulatory genes induced during TB-DM2 comorbidity, and their reliable protein-protein interaction network was described. Furthermore, the potential molecular patterns connected to them and the DEG induced in PDM2 are potential biomarkers for TB susceptibility in PDM2 patients.

Declarations

Declaration of interest: none.

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Consent to participate: Informed consent was obtained from all individual participants included in the study.

Authors' contributions: Elena Jaime-Sánchez: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data Curation, Writing, Original draft, Visualization. Edgar E. Lara-Ramírez: Methodology, Software, Formal analysis, Data Curation, Writing - Review & Editing; Juan Ernesto López-Ramos: Investigation, Resources, Data Curation, Writing - Review & Editing. Elsy Janeth Ramos-González: Formal analysis, Resources, Data Curation, Writing - Review & Editing. Ana Laura Cisneros-Méndez: Validation, Investigation. Juan José Oropeza-Valdez: Resources, Writing - Review & Editing. Roberto Zenteno-Cuevas: Resources, Writing - Review & Editing. Gerardo Martínez-Aguilar: Resources, Writing - Review & Editing. Yadira Bastian: Resources, Writing - Review & Editing. Julio Enrique Castañeda-Delgado: Resources, Writing - Review & Editing. Carmen Judith Serrano: Review &

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Figure Captions

Fig. 1 Identification of specific DEG in patients with PDM2 and TB-DM2. Venn comparative diagrams were made from Inter Genic Comparisons 1: a) induced and b) repressed genes and from Inter Genic Comparison 2: c) induced and d) repressed genes. Differentially expressed genes were identified in each study group with an absolute value of logFC> 2 and p <0.05 concerning the CTRL group from an Empirical Bayes analysis (moderate t-test). Venny 2.0 was used to obtain this figure. logFC: logarithm of the Fold Change

Fig. 2 Heat map of 446 DEG expression levels in the TB-DM2, TB, PDM2, DM2, and CTRL groups. Cluster analysis in 5 groups is shown in the columns, and the 446 genes analyzed are shown in the rows. The values of aver exp for each gene were clustered with the Euclidean method using the R program. The z-scale is shown on the upper left. Induced genes are represented with red, and repressed genes are represented with green. Cohort values p <0.05 and logFC> 1were obtained from empirical Bayes analysis (moderated T-test). logFC: logarithm of the Fold Change

Fig. 3 Prediction of KRG from molecular patterns: Resume for OSM, PRKCD, and SOCS3.
Networks of consensus terms are shown between induced DEG in PDM2 and TB-DM2. DEGs induced in PDM2 are shown as orange diamonds, while DEGs induced in TB-DM2 are represented as purple diamonds. The larger diamonds show key regulatory genes selected for microarray validation using RT-qPCR. The network visualization was made with the Cytoscape 3.5.1 program. KRG: Key regulatory genes

Fig. 4 OSM, PRKCD, and SOCS3 are reliable KRG. RT-qPCR assays evaluated OSM, PRKCD, and SOCS3 expression levels. The relative expression of a) OSM, b) PRKCD and c) SOCS3 is shown in the test set of the CTRL (n=17), DM2 (n=19), PDM2 (n=15), TB (n=9), and TB-DM2 (n=18) groups. Relative expression was calculated with the $2^{-\Delta\Delta Ct}$ equation.[21] Statistical analysis was performed on graph

Pad 6.0 with Kruskal Wallis and Dunn post hoc tests. *p <0.05, **p<0.01 with 95% of confidence interval. KRG: key regulatory genes

Fig. 5 Protein-Protein Interaction between OSM, PRKCD, SOCS3, IL6ST and STAT3 in humans using the STRING platform. The experimentally determined interactions are shown with pink edges. PPI enrichment p-value: 1.14e-07.

Supplementary Fig. 1 Signaling pathway patterns of combined DEGs of PDM2 / TB-DM2 and KRG identification. Induced (a) and repressed (b) genes.

Supplementary Fig. 2 Protein-protein interaction enrichment of OSM, PRKCD, SOCS3, IL6ST and STAT3 (the last two were included to shorten the interactome). The analysis was performed with InBioDiscover™

Tables

Table 1 Enrichment analyzes the signaling pathways associated with the differentially expressed genes induced in the PDM2 and TB-DM2 groups.

PDM2			**TB-DM2**		
Term	**Combined score**	**Genes**	**Term**	**Combined score**	**Genes**
Hematopoietic cell lineage	7.86*	*GP9, NFE2, GATA1*	TNF signaling pathway	19.86	*SOCS3; MMP9; IL18R1; CREB5*
Complex I biogenesis	7.49	*NDUFAF3, ECSIT, NT5M, SLC25A37*			
Hemostasis	6.86	*GP9, NFE2, KLC3, GATA1*	OSM Signaling Pathway	17.49	*SOCS3; PRKCD; OSM*
Cellular respiration	6.39*	*NDUFAF3, ECSIT, PRDX5*			
Interaction between L1 and Ankyrins	5.19	*ANK1**	IL-6 family signaling	16.97*	*SOCS3; OSM; IL6R, PRKCD*
TP53 Activity	5.18*	*PLK3, STK11, PRDX5*			
Nucleic acid metabolism	4.81*	*GMPR, NT5M*	IFNγ signaling	15.03*	*SOCS3; PRKCD; TRIM25; GBP2*
Clotting Cascade and Platelet activation	4.56*	*GP9, NFE2, KLC3, GATA1*			
Synthesis of glycosylphosphatidylinositol (GPI)	4.42*	*DPM2*	Adipogenesis	11.91	*SOCS3; NAMPT; OSM*
Cell Cycle	4.29*	*CDC34, PLK3, STK11, PRDX5*			
mTOR signaling pathway	3.88*	*STK11*	Jak-STAT signaling pathway	11.33	*SOCS3; OSM; IL6R*
Disease and infection by bacteria and viruses	3.71*	*KLC3, RILP, MRC2, PLK3, CDC34*			
Immune System	3.64*	*MRC2, TREML1, KLC3, CDC34, ECSIT, RILP*	Insulin Signaling Pathway	9.63*	*SOCS3; PRKCD; CREB5; SLC2A14, PFKL*
IL-1 signaling pathway	3.70*	*ECSIT*			
Kinesins	3.52	*KLC3*	Legionellosis	9.57	*CR1; NAIP*
Cancer	3.33*	*ANK1, PLK3, STK11*	IFN α/β signaling	9.31	*SOCS3; GBP2*
Muscle contraction	2.90*	*TCAP, RGS10*	AGE/RAGE pathway	9.25	*PRKCD; MMP9*
Antigen processing & presentation	2.87*	*MRC2, CDC34, KLC3, RILP*	Focal Adhesion-PI3K-Akt-mTOR-signaling pathway	8.91	*OSM; IL6R; CREB5*
N-Glycan biosynthesis	2.85*	*DPM2*			
Intraflagellar transport and assembly of cilium	2.62*	*IFT172*	Estrogen signaling pathway	8.83*	*PRKCD; MMP9; CREB5; TRIM25*
Signaling by Rho GTPases	2.60*	*KLC3*			
Toll-Like Receptor Pathway	2.59*	*ECSIT*	EPH-Ephrin signaling	8.69	*ARPC5; MMP9*
Protein Metabolism	2.59*	*DPM2, ANK1, DNAJB2, UBXN6, CDC34*	IL-3 Signaling Pathway	8.19	*SOCS3; MMP9*

Longevity regulating pathway - mammal	2.54	*STK11*	Fcγ R-mediated phagocytosis	7.4*	*PRKCD; ARPC5*
NF-kB activation	2.54*	*CDC34, ECSIT*			
PI3K-Akt signaling pathway	2.52*	*SKT11*	IL22 Soluble Receptor Signaling Pathway	4.93	*SOCS3*
ECM-receptor interaction	2.48	*GP9*			
Vesicle transport and vesicles	2.41*	*MRC2, RILP, KLC3, ANK1, PRDX5*	Role of ERBB2 in Signal Transduction and Oncology	4.2	*IL6R*
Metabolism of lipids and lipoproteins	2.38*	*THEM5, STK11*			
Metabolic pathways	2.21*	*DPM2, NT5M, SLC25A37, STK11, THEM5, NDUFAF3, GMPR, ECSIT*	Angiogenesis	3.4	*MMP9*
Insulin cascade	1.94*	*STK11, PLK3*	Regulation of PGC-1a	3.04	*SLC2A14*
MAPK signaling pathway	1.62*	*ECSIT*	IL-9 Signaling Pathway	2.99	*SOCS3*
Gene Expression	1.30*	*PLK3, STK11, PRDX5*	IL12 and Stat4 Dependent Signaling Pathway in Th1 Development	2.88	*IL18R1*
Developmental Biology	1.23*	*IFT172, ANK1*			
Signaling by VEGF	1.06*	*AXL*	Phosphoinositides and their downstream targets	2.64	*PFKL*
Signal Transduction	0.58	*KLC3, STK11, AXL, IFT172, RGS10*			
GPCR downstream signaling	0.50*	*RGS10*	Inhibition of Matrix Metalloproteinases	2.24	*MMP9*

*Average of the *combined score* between consensus pathways from the Keeg, Reactome, Wikipathways, and/or BioCarta databases. Number of genes induced in PDM2 and TB-DM2 that were enriched: 44 and 33

Supplementary Table 1 Clinical characteristics of the microarray and RT-qPCR populations evaluated in this study

	Study group					
	CTRL	**DM2**	**PDM2**	**TB**	**TB-DM2**	**p**
N						
Microarray	4	4	4	4	4	Does not
qPCR	17	19	15	9	18	apply
Age (years)						
Microarray	39.2 ± 4.0	51 ± 7.7	50.2 ± 5.0	31.2 ± 14.4	50.2 ± 8.7	0.061
qPCR	29 ± 10.8	48 ± 8.4	51.3 ± 5.9	28.3 ± 10.9	45.1 ± 8.8	< 0.0001
Sex (M/F)						
Microarray	2 / 2	2 / 2	2 / 2	3 / 1	3 / 1	0.87
qPCR	7 / 10	12 / 8	6 / 9	6 / 3	16 / 2	0.023
Glucose (mg/dl)						
Microarray	86.7 ± 8.3	191.5 ± 96.7	273.5 ± 40.5	90.5 ± 23.5	307 ± 171.7	0.0082
qPCR	89.5 ± 9.06	146.2 ± 47.9	235.9 ± 90	89.8 ± 14.9	233.4 ± 109.5	< 0.0001
HbA1c (%)						
Microarray	5.5 ± 0.3	7.7 ± 0.9	11.0 ± 0.6*	5.8 ± 2.9	11.6 ± 7.2	0.0077
qPCR	5.4 ± 0.4	7.6 ± 0.8	11.0 ± 0.8	5.8 ± 2.6	11.4 ± 6.3	< 0.0001

HbA1c levels were only available for seven and eight patients with TB and TB-DM2, respectively.

*p<0.05 in comparisons of the chi-square or Dunn's post-test. Mean ± SD are shown.

Supplementary Table 2 Sequences of the oligonucleotides used to amplify PRKCD, SOCS3, OSM, and HPRT in the test set by RT-qPCR

Gene	Access number	Forward oligonucleotide	Reverse oligonucleotide	Product size (bp)
OSM	NM_020530	GAAGCCCGTCTTGGGTCCTC	TCTGAGACCCCCTCTAGGAGA	100
PRKCD	NM_006254	CCTACAGCGACAAGAACCTCA	TCCAGGAGGTGCTCGAATTT	91
SOCS3	NM_003955	AGCGATGGAATTACCTGGAACA	TCCAGCCCAATACCTGACAC	109
HPRT	NM_000194	TGACCTTGATTTATTTTGCATACC	CGAGCAAGACGTTCAGTCCT	73

The oligonucleotides were acquired by Integrated DNA Technologies, Inc.

Supplementary Table 3 Aver Exp of 446 genes between the 5 microarray population groups.

GENE NAME	TB-DM2	TB	PDM2	DM2	CTRL	GENE NAME	TB-DM2	TB	PDM2	DM2	CTRL
TRBC1	9.48	9.32	11.29	10.51	10.84	*SNHG6*	8.43	8.35	8.97	9.12	9.37
RPL36A	8.97	9.11	7.97	9.35	9.58	*NINJ2*	7.37	6.89	8.47	7.64	8.24
CD5	7.03	6.83	7.4	7.88	7.84	*ERV18-1*	5.6	5.72	4.9	5.91	6.02
APOBEC3A	7.65	6.52	6.74	7.61	6.27	*LGR6*	12.12	12.14	11.07	12.1	12.15
GBP5	9.14	10.11	7.34	7.55	7.19	*NPM1P8*	6.85	6.87	7.07	7.77	8.03
C19orf59	7.61	7.42	6.87	7.08	6.04	*ANK1*	7.43	7.32	8.89	7.33	7.74
SCARF2	9.18	8.57	9.57	8.41	8.42	*RPUSD2*	5.43	6.37	7.14	6.6	6.8
SMOX	7.5	7.52	8.34	7.24	7.33	*IFI44*	6.92	7.51	5.64	6.55	5.88
RPSAP9	8.68	8.79	10.04	9.63	10.06	*CDC34*	10.03	9.95	10.92	10.07	9.85
RHOH	6.21	6.08	7.1	7.07	7.09	*RPL18A*	10.78	10.97	11.85	11.66	11.95
RPL7AP11	7.67	7.82	8.34	8.44	8.87	*RPL21P131*	10.91	10.77	9.99	10.91	11.1
RPL15P	9.79	10.07	11.27	10.69	10.99	*GYPC*	10.15	10.17	11.61	9.91	10.98
SKAP1	7.24	6.98	8.11	7.74	8.19	*ELOF1*	7.04	6.9	8.25	6.95	7.08
RPS2P	9.88	10.06	11.47	10.65	11.16	*MEX3D*	8.8	8.57	9.99	8.31	8.67
RPS2P	11.85	11.99	13.3	12.5	12.93	*RPS10P*	10.79	10.89	11.94	11.4	11.81
CD19	5.94	6.2	6.82	6.95	6.94	*IL6R*	8.01	7.51	7.38	7.49	7
FAM102A	7.1	6.98	8.43	8.37	8.56	*A_24_P662366*	8.15	8.21	6.07	8.09	8.53
NELL2	5.3	5.25	5.83	6.08	6.35	*TRAC*	8.52	8.97	8.92	9.43	9.79
PDZK1IP1	8.34	7.72	9.21	8.22	8.97	*CAMP*	8.32	8.37	7.42	7.9	6.83
SCARF1	6.79	6.68	5.64	5.7	5.59	*RP5-862P8.2*	8.61	8.46	7.26	8.68	8.34
PGLYRP1	6.77	6.38	6.31	6.16	5.73	*JAK2*	6.63	6.94	5.64	6.07	5.85
RPS2	11.51	11.73	12.48	12.4	12.72	*MT1B*	8.02	8.72	8.4	8.15	7.7
LCE1E	7.1	7.12	5.99	7.3	7.66	*RPS17P*	8.03	7.9	7.18	8.22	8.77
C1orf229	9.91	9.51	10.56	9.41	9.43	*CD274*	6.03	6.34	4.84	5.02	4.83
RPL29	8.84	9.02	9.99	9.67	10.08	*NT5M*	8.19	8.76	9.23	7.94	8.04
MMP9	10.88	10.16	9.91	9.76	9.35	*C1QB*	5.86	6.65	5.36	4.99	5
LCK	7.07	6.98	8.12	7.69	8.06	*SHISA5*	10.95	11.12	10.15	11.42	11.5
ISG15	9.11	9.87	8.1	8.56	8.41	*CD8A*	8.03	7.8	8.84	8.29	8.94
RPSAP58	8.96	9.02	9.95	9.79	10.25	*A_33_P3279456*	12.31	12.46	11.14	12.26	12.42
RPLP0	9.7	9.75	10.81	10.71	10.98	*BIRC3*	6.75	6.85	7.17	7.77	7.78
LOC729451	5.6	5.71	6.65	5.43	5.52	*CASP1*	7.32	7.93	6.18	6.7	6.41
RPL7P59	9.04	9.1	9.53	9.72	10.09	*RSAD2*	7.36	8.28	5.75	6.92	5.81
A_33_P3266614	8.76	8.29	9.6	7.87	8.1	*RPS4XP21*	5.93	5.68	4.89	6.01	6.21
RPL5P30	8.16	8.08	8.23	8.72	9.22	*RPL10A*	10.96	11.17	12.02	11.98	12.31
CLU	7.65	7.95	8.31	7.25	7.29	*BMP8B*	11.49	10.69	9.77	11.34	11.02
RPS4XP8	9.62	9.58	10.63	10.32	10.74	*HIST2H2AA4*	6.8	7.26	6.57	6.11	5.94
CREB5	7.58	7.24	6.68	6.99	6.48	*MT1E*	6.9	7.56	7.26	6.98	6.48
IFI44L	5.35	6.22	4.86	5.69	5.03	*TMED7-TICAM2*	8.07	7.91	6	7.96	7.59
RPL32P	7.34	7.39	6.76	7.55	7.94	*RPS21*	11.79	11.78	12.8	12.49	12.82
RPL18AP	9.03	9.23	10	9.98	10.35	*NRGN*	10.61	10.68	11.06	9.94	10

STAT1	7.8	8.26	6.81	7.19	6.7	*KRTAP5-14P*	8.21	8.36	6.8	9.04	9.23
OCIAD2	6.67	6.56	7.45	7.4	7.7	*FBL*	9.85	9.84	10.92	10.7	10.98
RPS6P	8.76	8.64	8.75	9.42	9.79	*RPL21P*	10.59	10.51	9.85	10.82	11.08
A_33_P3376836	8.73	8.54	7.09	8.62	8.37	*BATF2*	7.57	8.15	5.41	5.62	5.12
A_33_P3399181	7.6	7.34	8.35	6.99	7.12	*RPS3*	8.85	9.01	9.48	9.8	10.35
EPSTI1	5.91	6.36	5.14	5.26	5.11	*RPS16*	11.43	11.61	12.48	12.33	12.56
TMEM149	8.51	8.76	8.95	8.29	7.75	*UCP2*	7.41	7.77	8.74	8.03	8.52
SPOCK2	8.32	8.33	10.08	9.6	9.81	*RPS4XP13*	7.87	7.86	8.35	8.66	9.05
RPL7P12	6.44	6.37	5.49	6.59	6.89	*FAM131C*	14.66	14.53	13.54	14.61	14.58
TNFSF10	7.58	8.39	6.81	7.3	7.1	*RPS3P6*	8.6	8.76	9.74	9.34	10.02
KIAA0040	8.56	8.44	8.44	7.93	7.13	*RPL7AP60*	8.42	8.54	8.86	9.2	9.68
LEF1	7.11	7.02	8.01	8.38	8.4	*RPL36AP*	7.52	7.59	6.82	7.64	7.93
GLTSCR2	8.33	8.15	9.64	8.96	9.3	*RPS12*	13.41	13.47	12.21	13.47	13.52
NFE2	11.57	11.19	12.35	11.01	11.34	*PRRG4*	5.69	6.15	5.23	5.25	5.14
IGHM	6.52	7.68	7.65	7.65	8.07	*EIF3E*	8.32	8.63	9.13	9.37	9.64
IL18R1	6.2	5.68	5.3	5.31	5.16	*LOC100130938*	6.93	7.01	7.56	6.58	6.19
SLC25A37	10.64	10.47	11.6	10.81	10.48	*RPL10L*	9.64	9.79	10.77	10.66	11.1
GNB2L1	8.92	9.08	10.25	9.7	10.12	*EVL*	8.5	8.7	9.44	9.36	9.63
KLRB1	6.99	6.86	8.49	7.27	8.38	*TOMM7*	9.75	9.34	10.46	10.13	10.46
RAB35	7.7	7.47	8.35	8.42	8.89	*NPM1*	7.21	7.17	7.3	8.07	8.27
KIAA1841	8.25	8.04	8.88	7.68	7.71	*TXNDC17*	6.25	6.26	5.26	6.78	7.17
IGHD	5.12	5.16	5.74	5.55	6.29	*ASGR2*	8.02	7.55	6.69	7.14	6.8
RPS19	10.82	10.89	11.93	11.53	11.86	*NACAP1*	9.13	8.99	8.35	9.29	9.5
SMARCD3	7.38	7.56	6.55	6.43	6.35	*RPL29P*	9	9.21	9.82	10	10.31
FIS1	8.06	7.89	9.47	8	8.36	*PVRIG*	8.25	8.26	9.33	9.07	9.26
EEF1A1	9.06	9.16	9.8	9.87	10.25	*GBP2*	9.02	8.88	7.24	7.53	7.32
RPL18AP	8.44	8.58	9.8	9.32	9.73	*NDUFB4P12*	6.33	6.46	5.6	6.45	6.61
TRIM25	9.02	8.55	7.99	8.8	8.01	*PPIAP80*	6.92	7.15	6.79	7.47	7.8
RPLP0P	8.5	8.48	9.86	9.3	9.64	*CD2*	9.18	8.88	10.02	9.9	10.17
TMEM8C	10.72	10.82	10.17	10.99	11.18	*GLA*	8.3	8.78	6.97	8.16	8.02
TCF7	6.35	6.21	7.5	7.41	7.48	*TCL1A*	7.72	7.94	9.26	9.34	9.48
MRC2	8.01	7.46	9.45	7.96	8.35	*PLSCR1*	6.33	6.43	5.39	5.77	5.39
NAIP	7.98	7.33	6.61	7.32	6.53	*C20orf141*	9.23	9.23	10.52	10.11	10.48
FAM100A	6.21	6.09	7.49	6.27	6.37	*IFI27*	7.01	8.39	6.96	7.39	6.73
MAL	7.88	7.57	9.15	8.98	8.86	*RPS23*	9.03	9.07	9.37	9.82	10.06
KIAA0427	6.37	6.39	7.36	6.01	6.32	*PFKL*	8.14	7.14	6.2	7.04	6.98
FRMD3	6.15	6.38	5.78	5.62	5.34	*RPL6P2*	7.48	7.33	5.75	7.85	8.19
RPL12P4	9.7	9.6	10.67	9.85	10.6	*LOC102724737*	10.45	10.58	11.38	11.26	11.58
SOCS3	7.16	6.45	6.04	6.01	5.71	*HLA-DOA*	7.12	7.82	8.07	8	8.28
BX284668.2	7.52	6.9	6.79	6.36	6.38	*RPSAP52*	7.83	7.98	8.82	8.74	9.19
RPL29P7	7.7	7.98	8.58	8.65	9.06	*PRSS36*	7.22	7.2	5.86	7.83	8.21
MT1L	7.86	8.67	8.4	8.07	7.48	*lnc-EGLN1-1*	6.49	6.7	5.69	5.82	5.65
LINC00926	5.9	5.93	7.24	7.19	7.25	*APOL2*	9.85	10.01	8.69	9.19	8.94

CMPK2	6.12	6.85	5.32	5.88	5.4	*PARP14*	7.9	7.67	6.37	7.32	6.48
RPL3P6	7.55	7.29	7.59	7.85	8.37	*RPS4XP17*	8.13	8.23	8.8	9.11	9.57
Scarna17	8.3	7.88	10.03	9.69	8.75	*POU2AF1*	6.72	7.04	7.4	7.68	7.75
HIST2H2AA4	11.28	11.41	10.27	10.07	9.61	*NPM1*	8.66	8.63	9.33	9.66	9.82
PRKCD	8.82	8.4	7.95	8.19	7.82	*RPL18P11*	7.9	8.06	8.88	8.84	9.16
IL7R	8.79	8.13	9.51	9.58	9.62	*KLC3*	6.67	6.69	8.38	6.51	7.23
RPS8P	9.53	9.59	10.42	10.27	10.57	*USP32*	6.01	6.15	5.31	6.55	6.74
RPS28P	8.99	9	10.13	9.71	10.04	*FAM224B*	10.55	9.64	8.56	10.35	9.87
LINC01000	7.19	6.25	6.63	6.91	6.18	*RPL18AP15*	9.09	9.36	9.84	10.08	10.4
SERPING1	5.96	6.47	5.17	5.18	5.08	*C5orf4*	9.05	8.71	9.57	8.89	9.73
RPS4XP20	9.9	9.91	10.8	10.65	11.06	*NOD2*	7.66	7.86	6.33	7.37	6.71
PRDX5	9.7	9.84	10.66	9.85	9.64	*EPRS*	6.57	6.86	7.05	7.49	7.61
RPL9P18	9.72	9.76	10.81	10.41	10.93	*TRPC6*	8.7	8.98	7.22	8.85	8.39
HLA-F-AS1	9.28	9.34	10.3	9.87	10.29	*RPS2P32*	9.97	10.27	11.5	10.96	11.48
GBP1	5.73	6.4	5.02	5.31	5.17	*PLEKHB1*	6.07	5.89	6.91	6.81	7.08
DDX60L	7	7.03	5.69	6.76	6.01	*EEF1B2*	10.54	10.51	11.22	11.26	11.57
EVI2B	9.29	9.12	8.48	8.7	8.22	*ST6GAL1*	7.5	7.75	8.23	8.58	8.54
CD177	6.44	6.25	5.77	5.36	5.22	*BTBD2*	10.27	10.19	7.9	10.24	9.97
NAMPT	10.14	9.15	8.99	9.37	8.37	*PML*	8.11	8.63	7.48	7.68	7.26
GP1BB	11.23	11.53	11.38	10.74	10.51	*SNHG8*	8.13	8.31	8.99	8.94	9.33
TNFAIP6	6.91	7.39	5.84	6.17	5.6	*LDHB*	8.94	9.01	9.93	10.19	10.47
XAF1	6.25	6.66	5.36	5.86	5.56	*IL2RB*	7.44	7.41	8.09	7.91	8.45
E2F2	8.69	8.07	10.22	9.65	9.47	*GATS*	12.18	12.06	9.81	12.29	12.08
AES	8.6	8.49	9.67	9.6	9.83	*A_33_P3304748*	7.58	6.05	6.35	6.64	5.95
IL32	7.81	7.76	9.11	8.2	9.11	*RPL27P*	6.7	6.61	5.92	7.04	7.22
VAMP5	6.79	7.68	6.74	6.25	6.08	*RPL7P44*	8.54	8.68	8.09	9.32	9.65
ICA1	6.5	6.65	5.77	6.59	6.86	*GTF2IP13*	9.57	9.24	7.41	9.69	8.93
ND2	10.03	9.62	11.24	9.9	10.22	*slc2a14*	9.37	8.96	8.44	8.78	8.09
SNORD3B-1	7.9	7.99	9.22	7.33	7.6	*hist1h4l*	8.83	8.69	10.16	8.92	9.02
PPA2	9.39	8.9	10.32	8.64	8.95	*SPSB4*	11.53	11.21	9.44	11.15	10.94
AMOTL1	8.19	8.27	7.06	7.99	8.26	*RPL14P3*	9.28	9.43	9.84	10.13	10.37
RNA5-8S5	7.86	7.75	9.95	9.24	9.01	*SGSM2*	10.6	9.67	7.76	10.63	9.76
RPLP1P	10.25	10.51	11.13	11.31	11.31	*STK11*	8.13	8.01	9.18	7.76	8.1
ZNF438	7.77	7.49	6.51	6.78	6.44	*C11orf75*	7.25	8.05	7.05	7.01	6.72
RPL30P	9.57	9.91	10.61	10.15	10.58	*RNA18S5*	7.63	7.55	9.12	7.43	7.55
OAS3	5.64	6.16	5.24	5.57	5.15	*PRKCQ-AS1*	6.26	6.18	7.12	7.26	7.37
GZMK	6.35	6.13	7.6	7	7.35	*TNFRSF25*	6.52	6.52	7.73	7.66	7.78
ARHGEF15	9.43	9.48	8.65	9.69	9.83	*RPS3AP36*	8.56	8.69	8.82	9.51	9.86
EPB42	7.68	7.52	7.89	8.39	8.88	*RPSAP69*	8.3	8.53	9.63	9.39	9.87
C1S	6.34	6.44	5.35	6.58	6.82	*RPL31P45*	6.25	6.24	5.38	6.26	6.59
RPS27P20	10.37	10.31	11.59	11.05	11.45	*ITM2A*	6.68	7.1	7.92	7.67	8.05
RPL15P20	8.59	8.89	9.48	9.54	9.83	*ANKRD9*	7.55	7.54	8.08	7.08	6.96
C12orf57	8.73	8.19	9.57	8.86	9.28	*RPL19P12*	10.22	10.39	11.41	11.08	11.43

RPL17P22	7.27	7.29	7.64	7.99	8.29	*A_33_P3407230*	9.45	9.66	7.71	9.39	9.64
TBXAS1	9.34	9.2	7.12	8.64	9.03	*RPL29P12*	9.4	9.49	10.49	10.22	10.63
RPL17	10.11	10.15	10.69	10.94	11.13	*A_33_P3415221*	10.51	10.57	9.99	11.04	11.25
NTNG2	8.02	7.33	7.43	7.36	6.78	*THC2691510*	7.86	7.76	9.4	7.7	7.92
RPL15P11	9.45	9.72	10.08	10.38	10.48	*OLFML2B*	11.67	11.39	9.71	11.68	11.36
RPS6P25	9.58	9.46	10.35	10.19	10.63	*TRIM44*	7.63	7.37	6.18	7.51	7.29
A_33_P3343605	7.48	7.85	5.39	7.35	7.36	*RPS17P2*	8.92	8.87	9.57	9.37	9.91
RPS10	10.87	10.97	12.04	11.52	11.94	*RPS2P28*	10.92	11.12	12.4	11.75	12.18
EIF3F	6.65	7	7.82	7.59	8.01	*RPS18*	10.56	10.72	11.95	11.62	11.87
SPATA21	6.01	6.12	5.29	6.28	6.65	*PI3*	9.85	10.21	9.09	9.74	8.96
LYL1	7.21	7.04	8.66	7.48	8.07	*ANXA6*	6.5	6.38	7.1	7	7.46
IFIT2	8.46	8.96	7.07	7.92	7.32	*HP*	7.63	7.05	6.52	6.78	6.2
DB462629	6.49	6.4	6.46	7.04	7.5	*LOC100131262*	6.29	6	6.96	6.2	5.92
MIR4651	12.47	12.03	13.26	11.69	11.95	*GNLY*	8.65	8.04	9.95	8.7	9.21
CMTM5	7.24	7.19	7.95	6.78	6.79	*SORT1*	6.49	6.97	5.94	6.3	5.92
RPL15	8.62	8.71	9.54	9.48	9.67	*NFYC-AS1*	7.73	7.33	8.73	7.09	7.25
TPT1P8	8.93	9.21	7.27	9.07	9.41	*IGHA2*	7.09	6.95	6.61	7.73	6.12
RPL13	11.55	11.51	12.52	12.3	12.51	*FLJ42705 fis*	6.66	6.62	6.53	7.3	7.84
AC060814.3	9.05	8.34	8.4	8.13	8.02	*JPH3*	10.55	10.45	10.99	11.34	11.57
A_33_P3519223	5.81	5.82	4.81	5.83	6.29	*CR1*	6.63	5.95	5.57	5.79	5.61
PDS5A	10.08	10.14	7.93	9.28	8.27	*EEF1A1P15*	8.15	8.41	9.21	8.9	9.42
RPL13AP	8.75	8.85	9.36	9.7	9.94	*AC004086.1*	11.03	11.05	12.01	11.67	12.06
HINT1	9.8	10	10.73	10.73	10.91	*RPL31*	7.35	7.43	6.72	7.85	8.2
CISD3	10.4	10.06	11.18	9.9	10.04	*PCNX*	7.96	7.65	6.62	7.8	7.65
A_33_P3319937	11.09	10.54	12.04	10.23	10.51	*SNX10*	7.43	7.5	6.47	6.63	6.49
STAT6	9.65	9.5	8.8	9.36	9.91	*AIM2*	7.82	7.86	6.02	6.38	5.9
LOC441268	6.93	6.06	6.15	6.42	5.86	*RPS4X*	11.08	11.14	12.07	11.83	12.23
RPL13AP3	11.29	11.36	10.99	11.77	12.18	*CCR7*	8.32	8.28	9.55	9.82	9.8
OSM	7.66	7.21	6.12	6.02	5.98	*RPS16P8*	9.76	9.77	10.49	10.55	10.83
TRIB1	9.36	8.92	8.26	8.14	7.88	*RPL29P2*	7.63	7.78	8.78	8.33	8.85
RPL35	9.53	9.57	10.81	10.21	10.59	*RPS3AP49*	7.94	7.96	7.78	8.79	9.25
EEF1A1P	10.12	10.25	10.98	10.99	11.19	*DHRS9*	5.91	6.94	5.25	5.48	5.42
RPS10P	9.84	10.04	11.43	10.64	11.13	*RPS27AP7*	10.83	10.81	11.78	11.49	11.85
RPL4	10.01	10.13	11.07	10.94	11.27	*RPS10P16*	10.83	10.91	11.92	11.32	11.84
ZBP1	6.97	7.15	6.3	6.38	5.73	*RPL35P*	9.09	9.24	9.95	10.02	10.34
HIST1H2AD	9.54	9.75	9.02	8.62	8.3	*SECTM1*	11.11	11.39	9.79	10.42	9.82
DNAJB2	9.55	9.09	10.64	9.09	9.62	*MAB21L2*	6.21	6.41	5.36	6.64	7.12
ETV7	5.69	6.33	4.85	4.91	4.81	*HMGN1P31*	6.34	6.59	5.63	6.92	7.23
RPL9P16	10.57	10.65	11.44	11.18	11.63	*RPL23AP46*	10.78	10.88	11.56	11.39	11.79
A_33_P3422712	8.12	7.92	8.86	8.88	9.37	*AGPAT9*	7.48	7.23	6.78	6.82	6.37
RPSAP53	7.8	8.07	8.69	8.95	9.47	*POLR2H*	7.95	7.94	6.77	7.56	8.04
RNU2-1	8.78	9.56	10.48	6.73	10.54	*CD6*	7.22	7.02	8.33	7.93	8.21
RPL31P	9.27	9.39	8.14	9.58	9.71	*RTP4*	6.61	7.43	6.2	6.18	6.15

GP9	8.96	8.91	9.73	8.56	8.46	*IFIT3*	7.97	8.68	6.17	7.06	6.39
LRRC75A-AS1	9.13	9.01	10.05	9.83	10.14	*GPR183*	6.04	5.82	6.68	6.87	7.13
HIST1H3J	10.84	10.88	9.14	10.36	10.17	*PTK2B*	10.23	9.09	7.37	9.87	9.07
NCR3	6.52	6.77	8.22	7	7.8	*LOC100130152*	7.81	7.79	7.04	7.81	8.29
ACTG1P4	10.56	10.61	9.45	10.65	10.75	*LHFPL2*	6.07	6.6	5.53	5.41	5.25
KIAA0664	6.62	6.47	6.75	7.15	7.71	*C6orf25*	8.44	8.72	9.09	8.1	7.91
RPL3	11.18	11.11	12.19	11.86	12.21	*EEF2*	8.33	8.48	9.41	8.95	9.53
DTX3L	7.03	6.78	6.2	6.34	5.89	*MPP1*	7.67	7.44	8.45	7.09	7.06
THEM5	5.29	5.64	6.83	5.98	5.57	*RPS27*	13.4	13.11	13.93	13.95	14.21
RPL7AP8	9.79	9.86	10.29	10.38	10.83	*AKIRIN2*	6.71	6.49	6.47	5.84	5.69
TMEM204	6.06	5.88	7.14	6.73	7.06	*MRGPRG*	6.42	6.49	7.7	6.17	6.64
P2RY14	6.57	7.43	5.22	5.5	5.46	*RPL18*	7.1	7.29	7.77	7.83	8.21
TUSC8	12.06	11.59	12.64	11.41	11.55	*PTGDS*	6.47	6.68	8.48	6.38	7.69
RPL17P33	8.35	8.3	7.8	8.93	9.27	*RGS10*	11.2	11.16	12.1	11.18	11.05
C16orf35	8.7	8.59	10.06	7.98	8.55	*RNVU1-18*	11.25	12.37	12.06	8.58	12.58
ECSIT	7.84	7.57	8.97	7.55	7.93	*RPL18P*	8.59	8.7	9.76	9.18	9.87
FGL2	7.68	7.93	6.38	7.29	6.81	*RPS20*	12.16	11.91	12.94	12.73	12.99
TREML1	6.77	7	7.33	6.38	6.32	*FCGR1B*	10.24	9.88	7.93	8.44	7.25
RPL10AP3	8.57	8.66	9	9.55	9.86	*EPB42*	9.21	9.26	10.96	9.49	10.22
GNB2L1P	8.33	8.58	9.74	9.23	9.59	*CD247*	7.1	7.07	8.1	7.93	8.29
TECPR2	7.66	6.91	6.75	7.08	6.32	*UBXN6*	8.92	8.69	10.2	8.46	9.04
AP000872.1	5.83	5.96	5.02	6	6.15	*TRBC1*	9.81	9.67	11.31	10.85	11.18
RPS3A	10.36	10.59	11.31	11.25	11.71	*RPL7P26*	9.57	9.74	10.63	10.35	10.67
SLK	6.69	6.78	5.49	6.47	6.58	*LDHB*	6.83	6.9	7.53	7.93	8.27
RABEPK	7.06	7.01	5.76	7.13	6.78	*RPL35P1*	9.5	9.42	10.56	10.08	10.6
G0S2	6.97	7.42	5.3	5.32	5.43	*RPSAP2*	8.78	8.98	10.15	9.61	10.17
DPM2	7.89	7.55	9.38	8.05	8.21	*IFIH1*	7.5	7.54	6.39	6.83	6.32
RPL5	9.82	9.91	10.37	10.65	10.98	*WARS*	7.67	8.38	6.81	6.85	6.61
OASL	7.37	7.97	6.9	7.03	6.72	*GATA1*	6.21	6.04	7.38	6.12	6.36
SLC38A1	6.56	6.45	6.97	7.45	7.48	*EEF1A1P22*	10.37	10.4	11.27	11.23	11.39
RPL10	11.39	11.55	12.8	12.32	12.63	*MT2A*	9.83	10.72	10.04	10.02	9.41
RPL9	11.16	11.3	11.71	11.83	12.28	*ATP8*	10.17	9.58	10.68	9.5	9.66
MAFB	8.73	8.46	7.97	8.08	7.68	*MYOF*	5.81	6.5	5.31	5.47	5.34
FSD1	6.92	6.84	6.71	7.51	8.08	*RNF32*	8.71	8.21	9.68	8.01	7.88
ARPC5	11.79	11.37	11.26	10.95	10.53	*ASPRV1*	7.13	7.09	5.79	6.26	5.72
OXER1	7.7	7.47	6.92	7.16	6.67	*RPL9P2*	7.9	7.82	6.63	8.2	8.7
PEBP1	7.78	7.68	8.29	8.43	8.78	*LAP3*	5.9	6.68	5.33	5.77	5.62
RPS24P14	8.07	8.21	6.97	8.19	8.37	*TFDP2*	9.46	9.97	11.5	10.14	10.07
RPL17P50	10.3	10.2	10.11	10.97	11.12	*RPS2P*	7.71	7.93	8.96	8.64	9.27
NEURL	8	7.95	7.01	8.7	8.87	*gas5*	8.93	8.65	9.87	9.62	9.93
RHBDF2	8.82	9.29	7.75	8.11	8.11	*RPL22P11*	7.78	7.71	7.14	8.28	8.49
RPS4XP16	9.78	9.68	10.66	10.38	10.79	*GMPR*	6.8	6.71	8.28	7.14	7.13
AC000120.8	7.27	7.27	6.05	8.1	8.46	*SEC14L3*	7.2	6.4	8.68	7	6.57

DIRC1	10.59	10.62	8.71	11.38	11.19	*RPL29P*	8.17	8.2	9.18	8.93	9.27
PVRL2	5.58	6.32	5.09	5.33	5.23	*SAMD9L*	7.61	7.72	6.43	6.94	6.33
SLC2A4RG	7.61	7.46	8.76	8.14	8.54	*KRTAP19-2*	7.01	6.66	6.71	7.55	8.05
ATP5A1	7.81	8.18	8.64	8.64	8.82	*RILP*	6.19	6.28	7.53	6.02	6.41
RPS5	11.51	11.8	12.59	12.27	12.56	*RPL12P14*	9.84	9.72	10.74	10.23	10.79
CEACAM1	7.28	7.42	5.63	6.23	5.74	*FAM26F*	6.66	7.47	6.36	6.17	6.05
RPL29P30	8.1	8.25	9.19	8.92	9.35	*PPIAL4A*	7.73	7.82	8.57	8.41	8.78
UNCX	11.59	11.3	12.91	10.98	11.25	*TGM2*	6.33	6.09	7.92	6.2	6.62
MT3	12.37	12.36	9.93	12.34	11.96	*IFI6*	8.38	8.72	7.33	7.61	7.52
PADI2	6.78	6.19	5.9	5.73	5.46	*ANKRD22*	5.31	5.83	4.73	4.8	4.81
RBM38	9.27	9.3	10.88	9.47	9.67	*KCNQ2*	9.03	8.34	7.81	7.98	7.85

The Aver Exp of 446 genes were obtained after the intergroup comparison of TB-DM2, TB, PDM2, DM2 and CTRL. The average of four measurements are show by group. Aver Exp with $P<0.05$ from Bayesian analysis.

Supplementary Table 4 Microarray parameters of TB-DM2 key regulatory genes and their PDM2 associated DEG

Group	*Gene Symbol*	*Description*	*FC*	*p*
TB-DM2	*OSM*	oncostatin M	5.36	0.033
	PRKCD	protein kinase C, delta	2.73	0.00066
	SOCS3	suppressor of cytokine signaling 3	4.27	0.0074
PDM2	*MRC2*	mannose receptor, C type 2	2.96	0.022
	KLC3	kinesin light chain 3	3.1	0.011
	CDC34	cell division cycle 34 homolog (S. cerevisiae)	2.96	0.014
	STK11	serine/threonine kinase 11	2.91	0.0061
	ECSIT	ECSIT homolog (Drosophila)	2.77	0.00029
	RGS10	regulator of G-protein signaling 10	2.96	0.0073

This genes were obtained from the IntGC2 and IntGC1 respectively.

Supplementary Table 5. OSM, PRKCD, and SOCS3 interaction confidence scores with IL6ST and STAT3.

Interaction	Confidence score
OSM - IL6ST	1
PRKCD - IL6ST	1
SOCS3 - IL6ST	1
PRKCD - STAT3	1
SOCS3 - STAT3	0.28

The protein-protein interaction network was created at https://inbio-discover.com/ with the entry list OSM, PRKCD, SOCS3, IL6ST, and STAT3 (see supplementary Figure 2). The confidence scores were copied from the edges between the corresponding nodes.

Figures

Figure 1

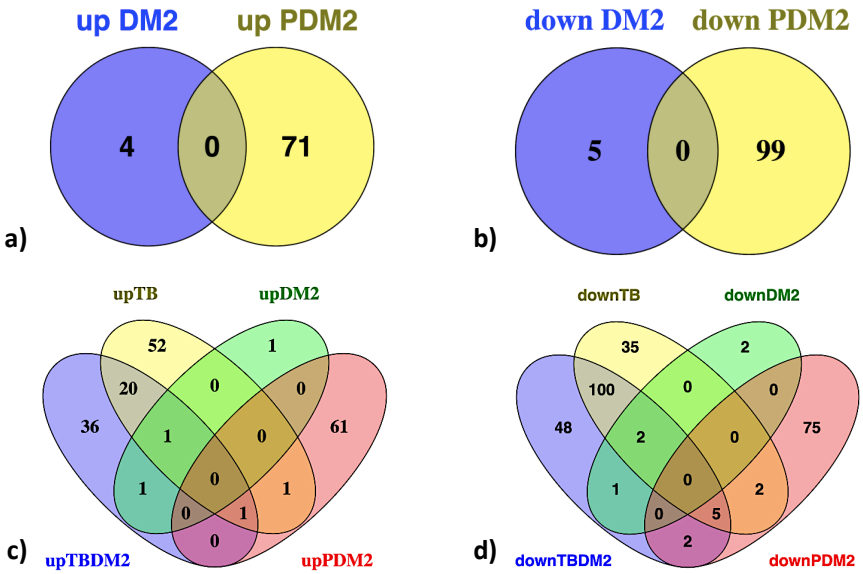


Figure 2

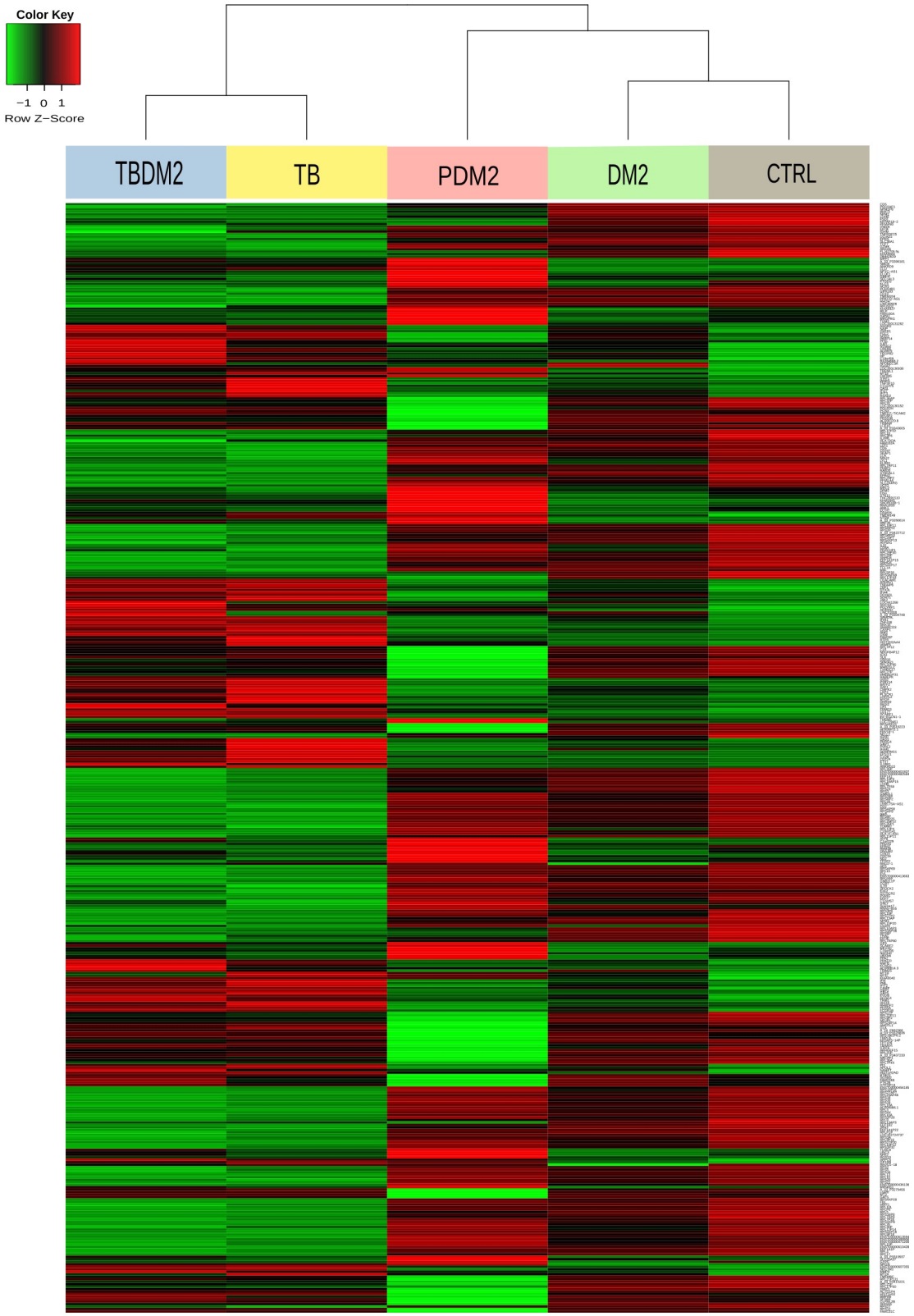


Figure 3

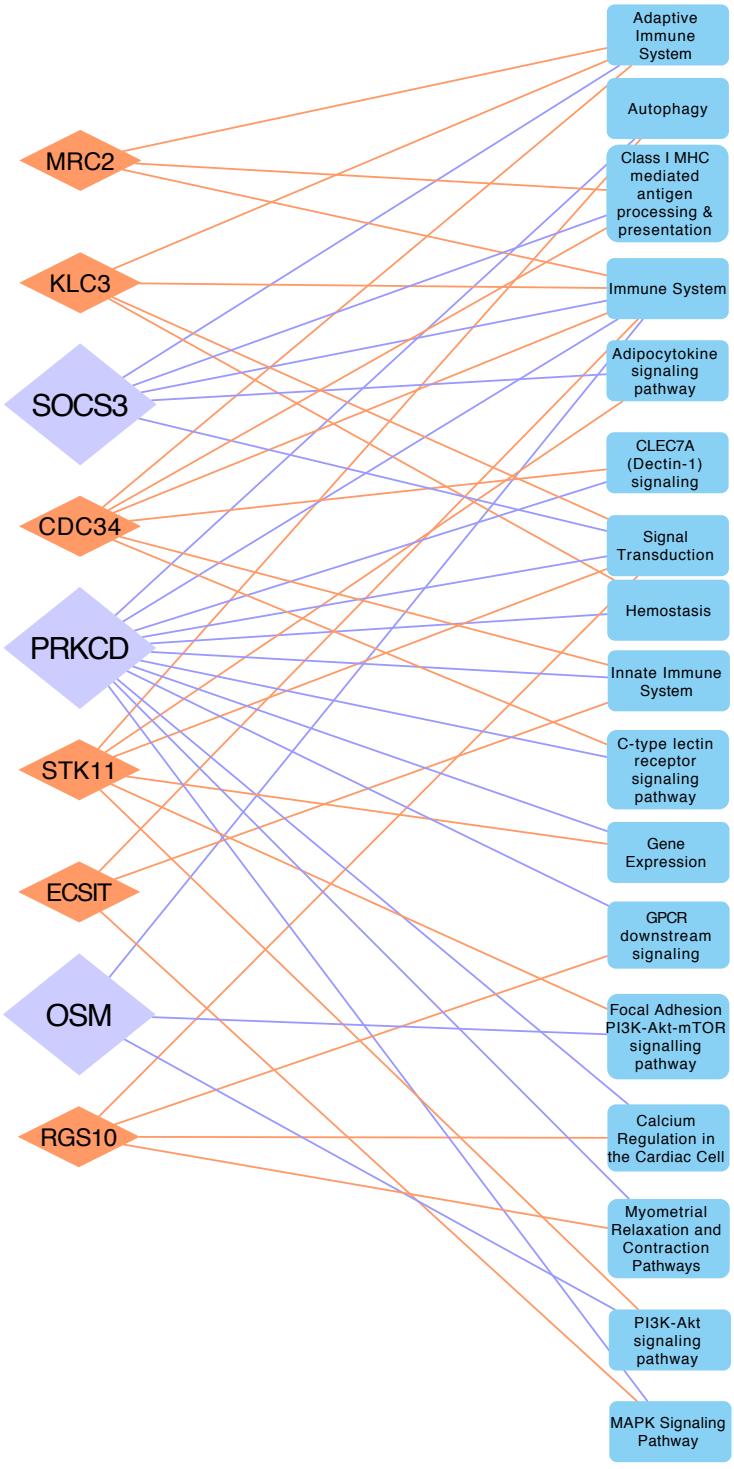

MRC2
KLC3
SOCS3
CDC34
PRKCD
STK11
ECSIT
OSM
RGS10
Adaptive Immune System
Autophagy
Class I MHC mediated antigen processing & presentation
Immune System
Adipocytokine signaling pathway
CLEC7A (Dectin-1) signaling
Signal Transduction
Hemostasis
Innate Immune System
C-type lectin receptor signaling pathway
Gene Expression
GPCR downstream signaling
Focal Adhesion PI3K-Akt-mTOR signalling pathway
Calcium Regulation in the Cardiac Cell
Myometrial Relaxation and Contraction Pathways
PI3K-Akt signaling pathway
MAPK Signaling Pathway

Figure 4

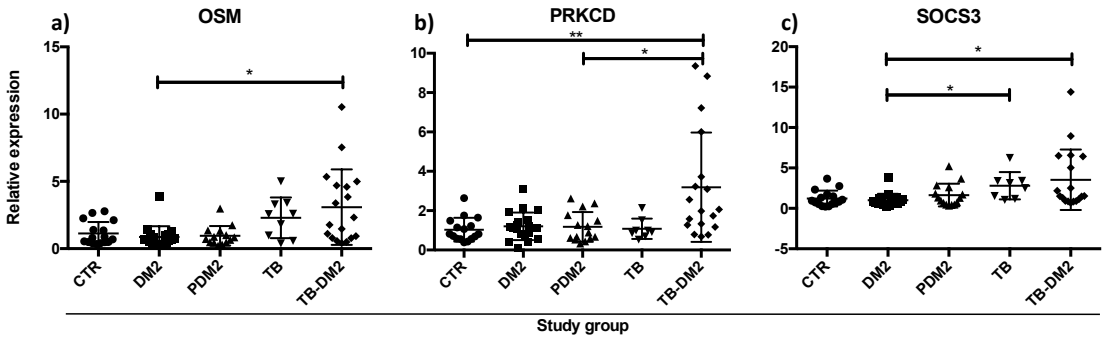
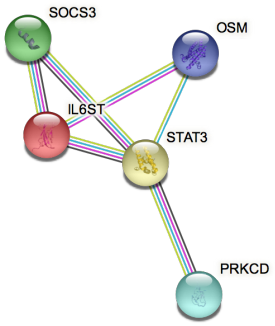
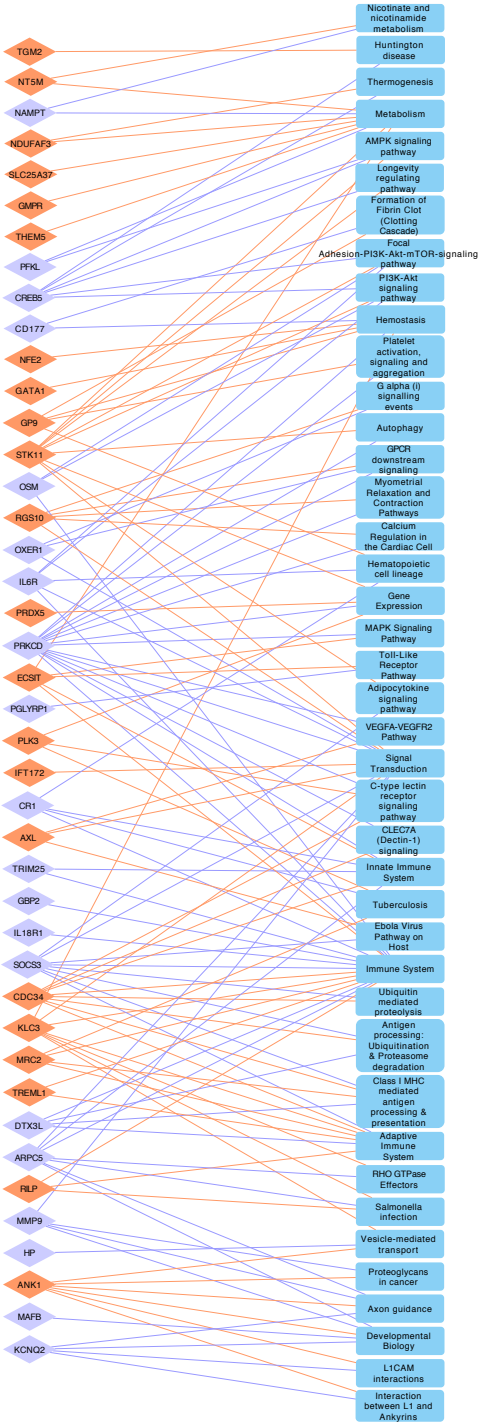

a)
OSM
b)
PRKCD
c)
SOCS3
Relative expression
Study group
CTR
DM2
PDM2
TB
TB-DM2
*
**

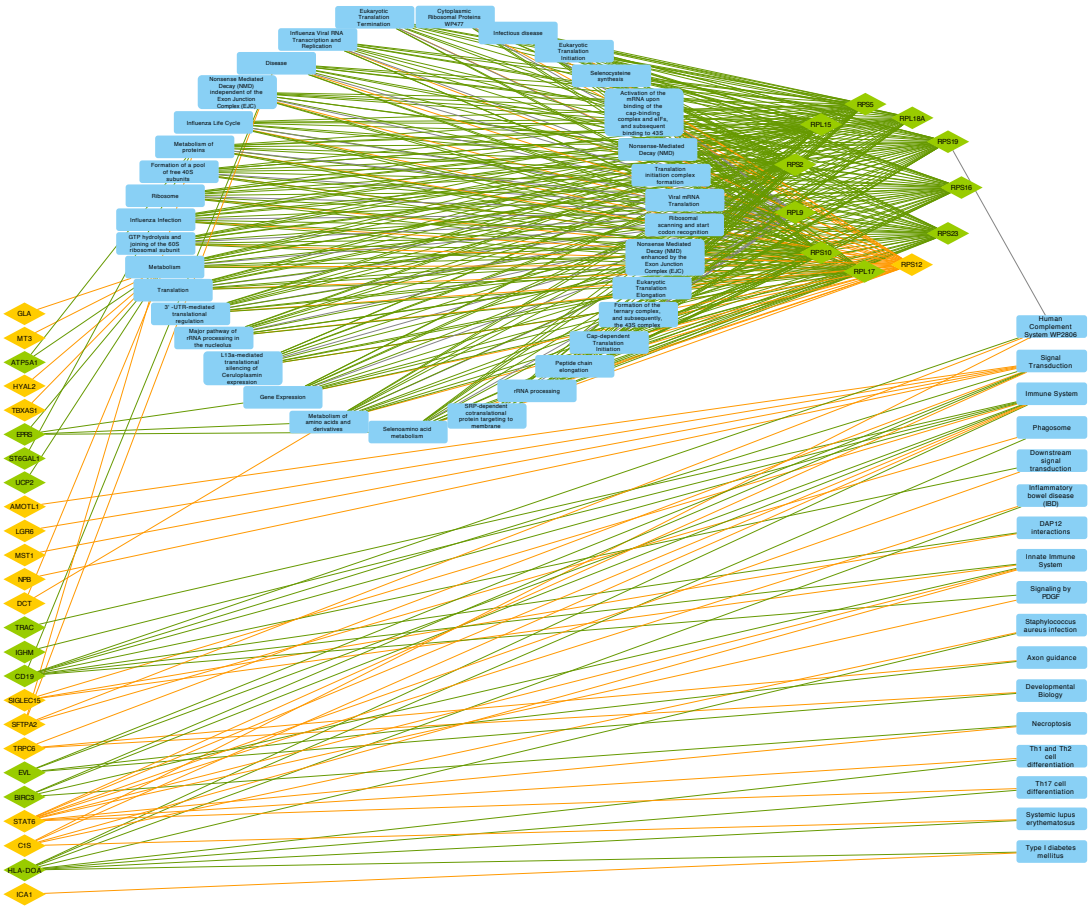
Figure 5



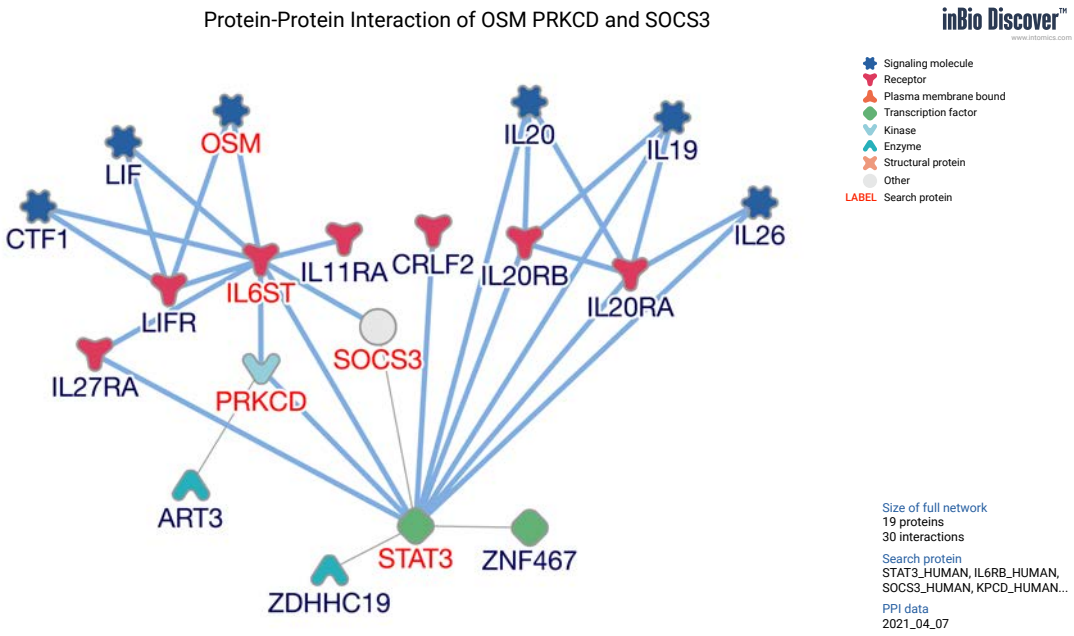
Supplementary Fig. 1a



Supplementary Fig. 1b



Supplementary Fig. 2



Annexes

Publications

Jaime-Sánchez Elena*, Lara-Ramírez Edgar E., López-Ramos Juan Ernesto, Ramos-González Elsy Janeth, Cisneros-Méndez Ana Laura, Oropeza-Valdez Juan José, Zenteno-Cuevas Roberto, Martínez-Aguilar Gerardo, Bastian Yadira, Castañeda-Delgado Julio Enrique, Enciso-Moreno José Antonio. Potential molecular patterns for tuberculosis susceptibility in diabetic patients with poor glycaemic control: A pilot study. Sent to Mol Genet Genomics, May 2022.

Jaime-Sánchez Elena*, Valtierra-Alvarado Monica Alejandra, Rivas-Santiago Bruno, Enciso-Moreno José Antonio, Serrano Carmen Judith. Mycobacterium tuberculosis infection is associated with a decreased suppressor of cytokine signaling 3 (SOCS3) expression in macrophages from poorly controlled patients with type 2 diabetes. Sent to Mol Biol Rep, Aug 2022.

Oropeza-Valdez Juan José, Moreira-Hernandez José de la Cruz, **Jaime-Sánchez Elena**, López-Ramos Ernesto, Lara-Ramírez Edgar E., López-Hernández Yamile, Castañeda-Delgado Julio Enrique, Enciso-Moreno José Antonio. Transcriptome analysis identifies oxidative stress injury biomarkers for Diabetic Nephropathy. Under review at Arch Med Res, Jan 2022.

López-Hernández Yamile, Lara-Ramírez Edgar E., Salgado-Bustamante Mariana, López Jesús Adrián, Oropeza-Valdez Juan J, **Jaime-Sánchez Elena**, Castañeda-Delgado Julio E, Magaña-Aquino Martín, Murgu Michael, Enciso-Moreno José A. Glycerophospholipid Metabolism Alterations in Patients with Type 2 Diabetes Mellitus and Tuberculosis Comorbidity. Arch Med Res. 2019. PMID: 31349956.

Macías-Segura N, Castañeda-Delgado J E, Bastian Y, Santiago-Algarra D, Castillo-Ortiz J D, Alemán-Navarro A L, **Jaime-Sánchez E**, Gomez-Moreno M, Saucedo-Toral C A, Lara-Ramírez Edgar E, Zapata-Zuñiga M, Enciso-Moreno L, González-Amaro R, Ramos-Remus C, Enciso-Moreno J A. Transcriptional signature associated with early rheumatoid arthritis and healthy individuals at high risk to develop the disease. PLoS One. 2018. PMID: 29584756.

***First author**

MGAG-D-22-00298 - Submission Notification to co-author - [EMID:860d93c8c5723605]

Molecular Genetics and Genomics (MGAG) <em@editorialmanager.com>

Lun 02/05/2022 02:01 PM

Para: Elena Jaime-Sánchez <nena_smile@live.com>

Re:"Potential molecular patterns for tuberculosis susceptibility in diabetic patients with poor glycaemic control: A pilot study"
Full author list: Elena Jaime-Sánchez; Edgar E. Lara-Ramírez; Juan Ernesto López-Ramos; Elsy Janeth Ramos-González; Ana Laura Cisneros-Méndez; Juan José Oropeza-Valdez; Roberto Zenteno-Cuevas; Gerardo Martínez-Aguilar; Yadira Bastian; Julio Enrique Castañeda-Delgado; Carmen Judith Serrano; José Antonio Enciso-Moreno
The submission id is: MGAG-D-22-00298

Dear MSc Jaime-Sánchez,

We have received the submission entitled: "Potential molecular patterns for tuberculosis susceptibility in diabetic patients with poor glycaemic control: A pilot study" for possible publication in Molecular Genetics and Genomics, and you are listed as one of the co-authors.

The manuscript has been submitted to the journal by Dr. PhD José Antonio Enciso-Moreno who will be able to track the status of the paper through his/her login.

Please could you confirm your co-authorship by clicking on the link below:
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Re: "Mycobacterium tuberculosis infection is associated with a decreased suppressor of cytokine signaling 3 (SOCS3) expression in macrophages from poorly controlled patients with type 2 diabetes."
Full author list: Elena Jaime-Sánchez; Monica Alejandra Valtierra-Alvarado; Bruno Rivas-Santiago; José Antonio Enciso-Moreno; CARMEN J. SERRANO

Dear Miss Jaime-Sánchez,

We have received the submission entitled: "Mycobacterium tuberculosis infection is associated with a decreased suppressor of cytokine signaling 3 (SOCS3) expression in macrophages from poorly controlled patients with type 2 diabetes." for possible publication in Molecular Biology Reports, and you are listed as one of the co-authors.

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Journal: Archives of Medical Research

Title: Transcriptome analysis identifies oxidative stress injury biomarkers for Diabetic Nephropathy

Corresponding Author: PhD Jose Antonio Enciso Moreno

Co-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

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Centro de Investigación en Ciencias de la Salud y Biomedicina (CICSaB)

PROGRAMA DE DOCTORADO EN CIENCIAS BIOMÉDICAS BÁSICAS

Tesis

"ANÁLISIS MOLECULAR Y FUNCIONAL DE LA RESPUESTA DE MACRÓFAGOS DE PACIENTES CON DIABETES A LA INFECCIÓN in vitro POR Mycobacterium tuberculosis"

M. en C. Elena Jaime Sánchez

31 Agosto 2022

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CONTENIDO

- Antecedentes
- Justificación
- Hipótesis
- Objetivo general
- Objetivos específicos
- Procedimiento experimental
- Resultados y discusión
- Conclusiones
- Publicaciones, cursos y divulgación científica

ABREVIATURAS

- TB: Tuberculosis
- TBL: TB Latente
- Mtb: *Mycobacterium tuberculosis*
- DM: Diabetes *mellitus*
- DM2: DM tipo 2
- DM2 pobre control: DM2 de pobre control glicémico
- CTRL: Controles aparentemente sanos
- GED: Genes Expresados Diferencialmente
- GR: Gen Regulador
- MDM: Macrófago derivado de monocito
- IPP: Interacciones Proteína-Proteína

4

Epidemiología de TB y DM

	Mundo				México		
	Casos	Incidencia /Prevalencia	Muertes	Causa de muerte	Casos	Incidencia /Prevalencia	Muertes
TB	9.9 millones	127*	1.3 millones	13a	31,000	24*	2,300
DM	537 millones ↑46%	9.3%	6.7 millones	9a	14.1232 millones	16.9%	184,384

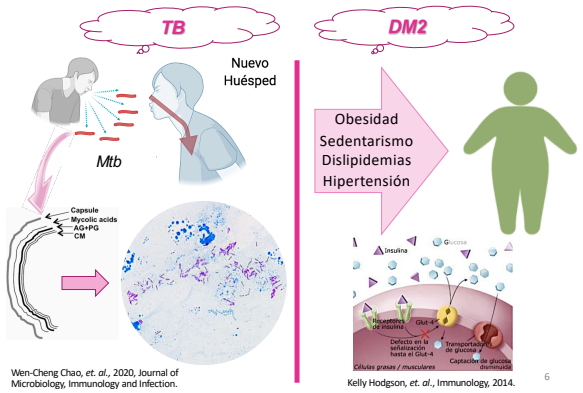
*número de casos por cada 100,000 habitantes.

- Infección por *Mtb*: 1/4 de la población mundial.

WHO TB REPORT, 2021; IDF DIABETES ATLAS, 9th / 10th Edition 2019/2021; Jean Jacques Noubiap, et. al., 2019, Lancet Glob Health.

5

Fisiopatología



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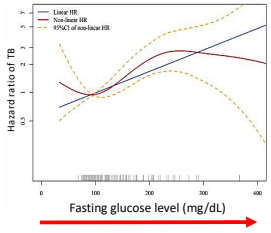
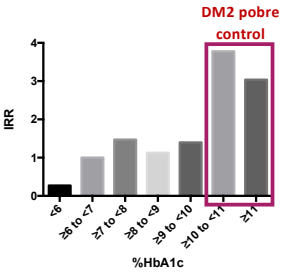
Kelly Hodgson, *et. al.*, Immunology, 2014.

6

HbA1c >10% significa más riesgo de TB





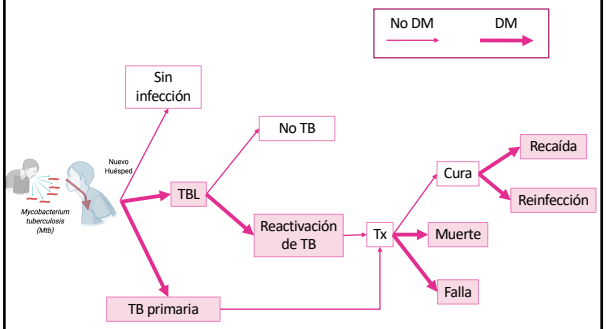
Pin-Hui L, *et.al.*, 2016, PLoS Med.

Julia A. Critchley, et. al., Diabetes Care, 2018.

7

7

Impacto de DM en la historia natural de TB



Modificado de Restrepo, *et. al.*, 2014, Diabetes Res Clin Pract. .

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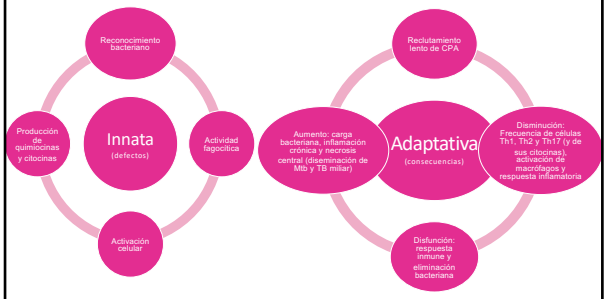
Impacto de DM en la respuesta inmune contra Mtb



Resultado:

- Mayor: susceptibilidad, reactivación, severidad, falla del tratamiento anti-TB y muerte.

Birhanu Ayelign, *et al.*, (2019), J. Immunol. Res.

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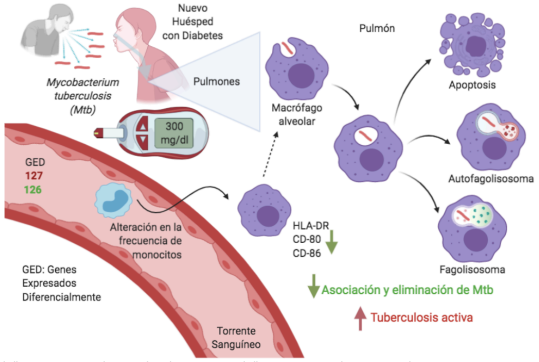
9

Impacto de DM en la respuesta inmune contra Mtb



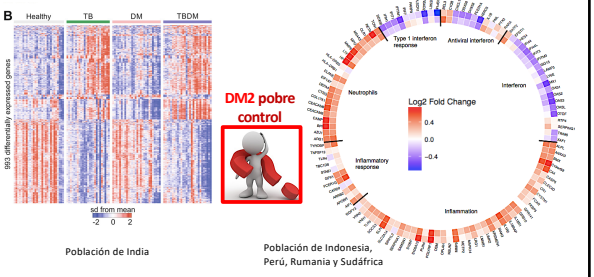
Nathella Pavan Kumar, *et. al.*, 2016, Tuberculosis Prada Medina, et. al., 2017, Sci Rep Nallely Lopez-Lopez, *et. al.*, 2018, Mem Inst Oswaldo Cruz

Nathella Pavan Kumar, *et. al.*, 2019, Immunology. Julia A. Critchley, et. al., Diabetes Care, 2018.

10

10

Transcriptómica de TB-DM2



Población de India

Población de Indonesia, Perú, Rumania y Sudáfrica

Prada-Medina, et. al., Sci Rep, 2017

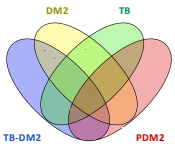
Eckold C, London School of Hygiene & Tropical Medicine (2018).

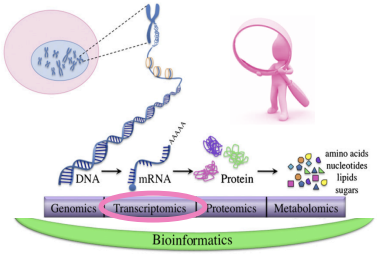
11

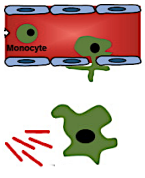
11

Asociación molecular de DM2 pobre control con TB-DM2

- La transcriptómica es una herramienta poderosa para identificar firmas de mecanismos inmunes en tuberculosis.







Judith M Fontana, et. al., 2012, Transl Res.

12

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Justificación

- El aumento de la población con DM2 representa una amenaza para la erradicación de TB.
- Conocer los mecanismos moleculares que participan en la susceptibilidad de los pacientes con DM2 con pobre control glicémico a desarrollar TB podría ayudar a controlar esta epidemia.
- Sin embargo, su investigación aún es limitada.
- La asociación de GR de TB-DM2 con DM2 con pobre control glicémico aportará información nueva, que ayude a explicar la falta de capacidad de los pacientes DM2 con pobre control glicémico de controlar la infección por Mtb.
- Así mismo, dirigir la evaluación de dichos GR a macrófagos DM2 pobre control ayudará a elucidar su papel durante la infección *in vitro con Mtb* en el desarrollo de TB-DM2 primaria.

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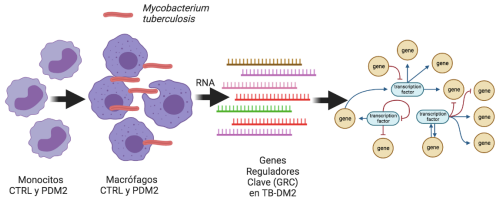
Hipótesis

- La alteración de la expresión de Genes Reguladores de TB-DM2 y DM2 con pobre control glicémico reduce la capacidad de los macrófagos de sujetos DM2 con pobre control glicémico para controlar la replicación de Mtb *in vitro*.



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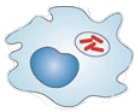
Objetivo general

- Identificar genes reguladores en pacientes DM2 con pobre control glicémico y TB-DM2 cuya interacción explique la incapacidad de los MDM para eliminar *Mtb in vitro*.



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Objetivos específicos

1. Microarreglo — Respuesta periférica

1. Identificar el transcriptoma de TB-DM2 y DM2 con pobre control glicémico mediante microarreglos de expresión.
2. Realizar el minado de datos del microarreglo de expresión mediante bioinformática e identificar genes reguladores de TB-DM2 asociados a DM2 con pobre control glicémico.
3. Validar el microarreglo de expresión mediante qPCR.

2. Ensayos *in vitro* — Respuesta local

4. Evaluar la capacidad de los macrófagos de pacientes con PDM2 para controlar la replicación de Mtb in vitro respecto al CTRL, mediante ensayos de UFCs.
5. Evaluar los niveles de traducción de proteínas en macrófagos de pacientes con PDM2 infectados *in vitro* con Mtb respecto al CTRL utilizando Citometría de Flujo.
6. Evaluar la ER de los genes reguladores de TB-DM2 en macrófagos de pacientes con DM2 con pobre control glicémico infectados in vitro con *Mtb* respecto al CTRL mediante qPCR.

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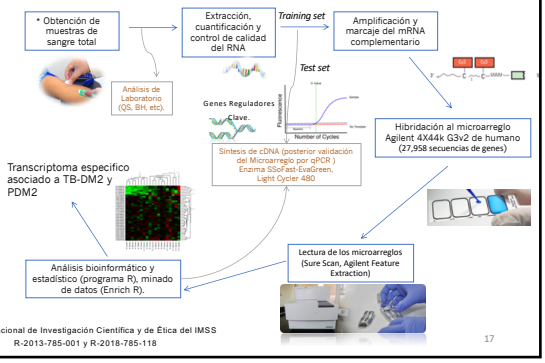
Identificación de genes reguladores mediante transcriptómica. Grupos: TB-DM2 , DM2 pobre control, TB, DM2 y CTRL



*Comité Nacional de Investigación Científica y de Ética del IMSS R-2013-785-001 y R-2018-785-118

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Criterios de inclusión para población de Microarreglos y qPCR.

Característica	Grupo de estudio				
	CTRL	DM2	DM2 pobre control	TB	TB-DM2
COMBE	-	-	-	+	+
PPD	-	-	-	NA	NA
BAAR y/o CULTIVO Y/O GENE EXPERT	-	-	-	+	+
DIAGNÓSTICO DE DM2	-	+	+	-	+
GLUCOSA EN AYUNO (mg/dl)	<126	>126	>126	<126	>126
%HbA1c	<6.4	6.5-8.9	>10	<6.4	>6.5

Carta de consentimiento informado. Edad: 18-65 años. CTRL: control; TB: tuberculosis; DM2: diabetes mellitus tipo 2 con adecuado control glicémico, DM2 pobre control: diabetes mellitus de tipo 2 con pobre control glicémico, TB-DM2: binomio; COMBE: contacto a Mtb; PPD: *purified protein derivative*; BAAR: bacilos ácido alcohol resistentes; (+): positivo; (-): negativo; NA: no aplica.

Comité Nacional de Investigación Científica y de Ética del IMSS
R-2018-785-118

Criterios de exclusión: Infección por VIH, Aplicación de PPD 8 semanas previas o menos, Pacientes tratados con fármacos anti-tuberculosis (con más de 1 mes de tratamiento), inmunosupresores o corticosteroides.

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Resultados y discusión

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Población de Estudio

Tabla 1. Características clínicas de la población de microarreglos y qPCR.

		Grupo de estudio					
		CTRL	DM2	DM2 pobre control	TB	TB-DM2	p
N							
	Microarreglo	4	4	4	4	4	No aplica
	qPCR	17	19	15	9	18	
Edad (años)							
	Microarreglo	39.2 ± 4.0	51 ± 7.7	50.2 ± 5.0	31.2 ± 14.4	50.2 ± 8.7	0.061
	qPCR	29 ± 10.8	48 ± 8.4	51.3 ± 5.9	28.3 ± 10.9	45.1 ± 8.8	< 0.0001
Sexo (H/M)							
	Microarreglo	2 / 2	2 / 2	2 / 2	3 / 1	3 / 1	0.87
	qPCR	7 / 10	12 / 8	6 / 9	6 / 3	16 / 2	0.023
Glucosa (mg/dl)							
	Microarreglo	86.7 ± 8.3	191.5 ± 96.7	273.5 ± 40.5	90.5 ± 23.5	307 ± 171.7	0.0082
	qPCR	89.5 ± 9.06	146.2 ± 47.9	235.9 ± 90	89.8 ± 14.9	233.4 ± 109.5	< 0.0001
HbA1c (%)							
	Microarreglo	5.5 ± 0.3	7.7 ± 0.9	11.0 ± 0.6*	5.8 ± 2.9	11.6 ± 7.2	0.0077
	qPCR	5.4 ± 0.4	7.6 ± 0.8	11.0 ± 0.8	5.8 ± 2.6	11.4 ± 6.3	< 0.0001

Los niveles de HbA1C estuvieron disponibles solamente para siete y ocho pacientes con TB y TB-DM2 respectivamente. *p<0.05 en las comparaciones de CHI cuadrada o del post-test de Dunn. Se muestra la media ± DE.

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Perfil transcripcional TB-DM2, TB, DM2 pobre control, DM2 y CTRL

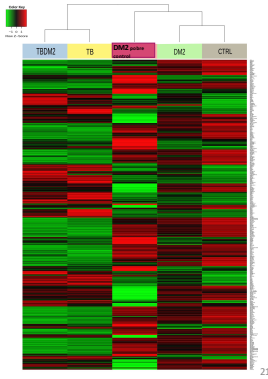

Figura 1. Mapa de calor de los niveles de expresión de 446 genes en los grupos TB-DM2, TB, PDM2, DM2, y CTRL. Se muestran los genes en las filas los valores promedio de expresión de cada grupo en las columnas. La "*z-scale*" se muestra en la parte superior izquierda. Rojo: genes inducidos. Verde: genes reprimidos. Valores de cohorte del análisis de Bayes empírico: p <0.05 y valor absoluto de FC >2. FC: "*Fold Change*"

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Identificación de GED en DM2 pobre control y TB-DM2

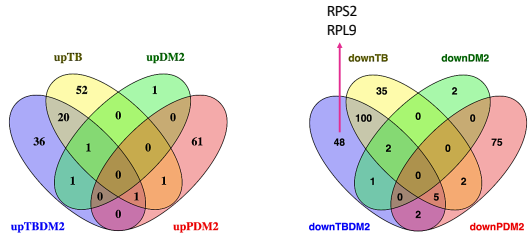

Figura 2. Los GED se identificaron con un valor absoluto de FC >2 y de p<0.05 para cada grupo de estudio con respecto al grupo CTRL mediante un análisis de Bayes empírico (t moderada). Genes inducidos (izquierda) y reprimidos (derecha). Se utilizó Venny 2.0 para generar esta figura. FC: Fold Change.

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Vías de señalización asociadas con DM2 pobre control y TB-DM2

Tabla 2. Análisis de enriquecimiento de las vías de señalización asociadas con los GED inducidos en PDM2 y TB-DM2.

DM2 pobre control			TB-DM2		
Term	Combined score	Genes	Term	Combined score	Genes
Hematopoietic cell lineage	7.86*	*GP9; NFE2, GATA1*	TNF signaling pathway	19.86	*SOCS3; MMP9; IL18R1; CREB5*
Complex I biogenesis	7.49	*NDUFAF3, ECSIT, NT5M, SLC25A37*	OSM Signaling Pathway	17.49	*SOCS3; PRKCD; OSM*
Hemostasis	6.86	*GP9; NFE2, KLC3, GATA1*	IL-6 family signaling	16.97*	*SOCS3; OSM, IL6R, PRKCD*
Cellular respiration	6.39*	*NDUFAF3, ECSIT, PRDM5*			
Interaction between L1 and Ankyrins	5.19	*ANK1**	IFNγ signaling	15.03*	*SOCS3; PRKCD; TRIM25; GBP2*
TP53 Activity	5.18*	*PLK3, STK11, PRDM5*			
Nucleic acid metabolism	4.81*	*GMPR, NT5M*	Adipogenesis	11.91	*SOCS3; NAMPT; OSM*
Clotting Cascade and Platelet activation	4.56*	*GP9, NFE2, KLC3, GATA1*			
Synthesis of glycosylphosphatidylinositol (GPI)	4.42*	*DPM2*	Jak-STAT signaling pathway	11.33	*SOCS3; OSM, IL6R*
Cell Cycle	4.29*	*CDC34, PLK3, STK11, PRDM5*			
mTOR signaling pathway	3.88*	*STK11*	Insulin Signaling Pathway	9.63*	*SOCS3; PRKCD; CREB5; SLC2A14, PFKL*
Disease and infection by bacteria and viruses	3.71*	*KLC3, RELP, MRC2, PLK3, CDC34 MRC2, TBX6M1, KLC3, CDC34,*			
Immune System	3.64*	*ECSIT, RELP*	Legionellosis	9.57	*CB1; NAIP*
IL-1 signaling pathway	3.70*	*ECSIT*	IFN-α/β signaling	9.31	*SOCS3; GBP2*
Kinesins	3.52	*KLC3*	ACE/BRADE pathway	9.25	*PRKCD; MMP9*
Cancer	3.33*	*ANK1, PLK3, STK11*	Focal Adhesion-PI3K-Akt-mTOR-signaling pathway	8.91	*OSM, IL6R, CREB5*
Muscle contraction	2.90*	*TCAP, RGS10*			
Antigen processing & presentation	2.87*	*MRC2, CDC34, KLC3, RELP*	Estrogen signaling pathway	8.83*	*PRKCD; MMP9; CREB5; TRIM25*
N-Glycan biosynthesis	2.85*	*DPM2*			
Intraflagellar transport and assembly of cilium	2.62*	*IFT172*	EPH-Ephrin signaling	8.69	*ARPC5; MMP9*
Signaling by Rho-GTPases	2.60*	*KLC3*			
Toll-Like Receptor Pathway	2.59*	*ECSIT*	IL-3 Signaling Pathway	8.19	*SOCS3; MMP9*
Protein Metabolism	2.59*	*DPM2, ANK1, DNAJB2, UBXN6, CDC34*			
Longevity regulating pathway - mammal	2.54	*STK11*	Fcγ R-mediated phagocytosis	7.4*	*PRKCD; ARPC5*
NF-kB activation	2.54*	*CDC34, ECSIT*			

*Promedio del "combined score" entre las vías de señalización consenso de las bases de datos Keeg, Reactome, Wikipathways, y/o BioCarta. DEG inducidos que fueron enriquecidos en PDM2 y TB-DM2: 44 y 33.

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Selección de GR a partir de la jerarquía ontológica y *pathways* de regulación relevantes en TB-DM2 y DM2 pobre control

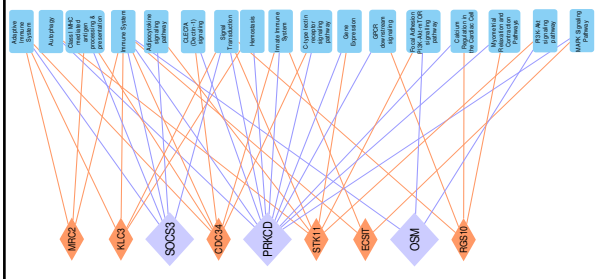

Figura 4. Predición de GRC a partir de patrones moleculares. Resumen de OSM, PRKCD y SOCS3. Se muestran los términos consenso de redes de los GED en PDM2 y TB-DM2. Los diamantes representan genes inducidos en PDM2 (naranjas) y TB-DM2 (morados). Los GRC seleccionados para validación del microarreglos mediante qPCR se representaron con diamantes grandes. Se utilizó el programa Cytoscape 3.5.1 para visualizar la red. GRC: Genes Reguladores Clave.

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Descripción de GR en TB-DM2 y los asociados con DM2 pobre control

Tabla 3. GRC de TB-DM2 asociados con los GED en PDM2 y sus parámetros en el microarreglo.

Grupo	*Gen*	*Descripción*	*FC*	*p*
TB-DM2	*OSM*	oncostatin M	5.36	0.033
	PRKCD	protein kinase C, delta	2.73	0.00066
	SOCS3	suppressor of cytokine signaling 3	4.27	0.0074
PDM2	*MRC2*	mannose receptor, C type 2	2.96	0.022
	KLC3	kinesin light chain 3	3.1	0.011
	CDC34	cell division cycle 34 homolog (S. cerevisiae)	2.96	0.014
	STK11	serine/threonine kinase 11	2.91	0.0061
	ECSIT	ECSIT homolog (Drosophila)	2.77	0.00029
	RGS10	regulator of G-protein signaling 10	2.96	0.0073

Estos genes se obtuvieron de las comparaciones intergénicas 2 y 1, respectivamente.

Tabla 4. Oligonucleótidos utilizados para amplificar PRKCD, SOCS3, OSM, y HPRT en el "test set" por qPCR.

Gen	*Número de acceso*	*Oligonucleótido sentido*	*Oligonucleótido antisentido*	*Tamaño del producto (pb)*
OSM	NM_020530	GAAGCCCGTCTTGGGTCCTC	TCTGAGACCCCCTCTAGGAGA	100
PRKCD	NM_006254	CCTACAGCGACAAGAACCTCA	TCCAGGAGGTGCTCGAATTT	91
SOCS3	NM_003955	AGCGATGGAATTACCTGGAACA	TCCAGCCCAATACCTGACAC	109
HPRT	NM_000194	TGACCTTGATTTATTTTGCATACC	CGAGCAAGACGTTCAGTCCT	73

Los oligonucleótidos fueron adquiridos de Integrated DNA Technologies, Inc.

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OSM, PRKCD y *SOCS3* son potenciales Genes Reguladores inducidos en TB-DM2

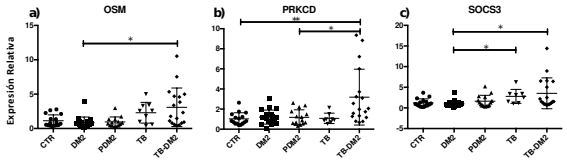

Fig. 4 OSM, PRKCD y SOCS3 son potenciales GRC y sus niveles de expression fueron evaluados mediante qPCR. La expression relativa de a) OSM, b) PRKCD y c) SOCS3 se muestra en los grupos CTRL (n=17), DM2 (n=19), PDM2 (n=15), TB (n=9) y TB-DM2 (n=18). La expression relativa se calculó mediante la ecuación de $2^{-\Delta\Delta CT}$. El análisis estadístico se realize en graph Pad 6.0 con la pruebas de Kruskal Wallis y post-test de Dunn. *p <0.05, **p<0.01 con 95% de intérvalo de confianza. GRC: genes reguladores clave

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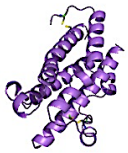
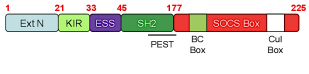
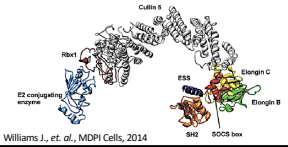
OSM, PRKCD y *SOCS3*



PDB

Salzer E., *et. al.*, J Clin Immunol, 2016.





Williams J., *et. al.*, MDPI Cells, 2014

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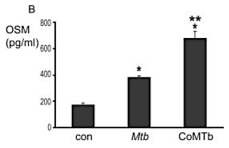
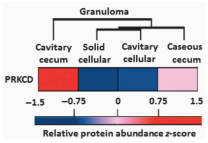
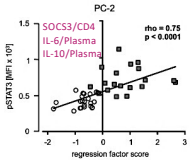
OSM, PKCδ y SOCS3 durante TB



Cecilia M. O'Kane, *et. al.*, Eur. J . Immunol, 2008.



SP Parihar, *et. al.*, Mu cosallmmunology, 2017.



Kirstin Harling, *et. al.*, Cellular and Molecular Immunology, 2017.

28

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OSM, PRKCD y *SOCS3*: Interacciones Proteína-Proteína

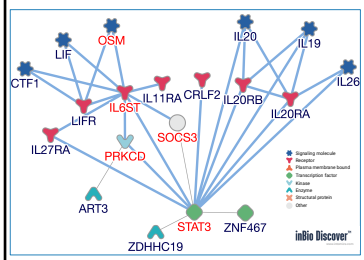

Figura 6. Enriquecimiento de Interacciones Proteína-Proteína de OSM, PRKCD, SOCS3, IL6ST y STAT3 (las últimas dos se incluyeron para acortar el interactoma). El análisis se realizó en InBioDiscover™.

Tabla 6. "*Confidence score*" de interacción de OSM, PRKCD y SOCS3 con IL6ST y STAT3.

Interaction	*Confidence score*
OSM - IL6ST	1
PRKCD - IL6ST	1
SOCS3 - IL6ST	1
PRKCD - STAT3	1
SOCS3 - STAT3	0.28

La red de Interacción Proteína-Proteína se creó en https://inbio-discover.com/ con la lista de entrada OSM, PRKCD, SOCS3, IL6ST y STAT3. Los "confidence scores " se copiaron de las aristas entre los nodos de los genes correspondientes.

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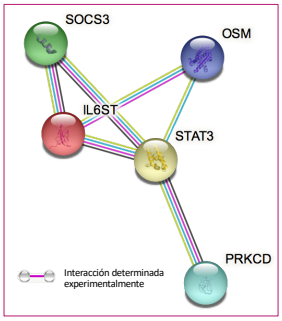
Asociación funcional de *OSM, PRKCD* y *SOCS3* (análisis exploratorio) Interacciones Proteína-Proteína / evidencia experimental



Combined score: >0.972

Figura 7. Interacciones Proteína- Proteína entre OSM, PRKCD, SOCS3, IL6ST y STAT3 en humanos de acuerdo con la plataforma STRING. Las interacciones que han sido demostradas experimentalmente se muestran con aristas de color rosa. Enriquecimiento PPI: p = 1.14e-07.

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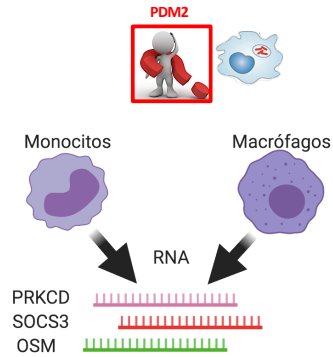
Los monocitos y macrófagos expresan *PRKCD*, *SOCS3* y *OSM*



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Modelo de infección de MDM con Mtb *in vitro*

Tabla 5. Características clínicas de los sujetos de estudio.

Características	CTRL	PDM2	P
N	10	9	NA
Años con Dx DM2	NA	6.01 ± 5.2	NA
Edad, años	42.3 ± 6.8	47 ± 8.5	0.20
IMC (kg m^{-2})	24.3 ± 2.7	26.24 ± 3	0.15
HbA1c (%)	5.1 ± 0.3	10.84 ± 1.9	< 0.0001****
Glucosa (mg dL^{-1})[a]	81.4 ± 10.9	239.8 ± 64.16	< 0.0001****
Colesterol (mg dL^{-1})[b]	151 ± 41.8	168.8 ± 55.3	0.49
HDL (mg dL^{-1})	55.1 ± 11.8	40.3 ± 9.3	0.013*
LDL (mg dL^{-1})	96.3 ± 25.5	101.9 ± 36.2	0.73
VLDL (mg dL^{-1})	18.4 ± 6.2	58.5 ± 42.3	0.019*
Triglicéridos (mg dL^{-1})[c]	87.4 ± 29.1	274.8 ± 204	0.0005***
Hipertensión, n (%)	0 (0)	4 (44.4)	NA

Las diferencias entre grupos se identificaron mediante "t-tests", excepto para la Glucosa[a], Colesterol[b] y triglicéridos[c], los cuales se compararon utilizando pruebas U de Mann-Whitney. Los datos de las variables cuantitativas se presentan como medias ± desviación estándar..
IMC, índice de masa corporal; HbA1c, % de hemoglobina glicosilada; HDL, lipoproteínas de alta densidad; LDL, lipoproteínas de baja densidad; NA, no aplica; VLDL, lipoproteínas de muy baja densidad. CTRL: sujetos control aparentemente sanos, PDM2: pacientes con diabetes tipo 2 de pobre control glicémico.

*Comité Nacional de Investigación Científica y de Ética del IMSS
R-2018-785-124 y R-2018-785-118

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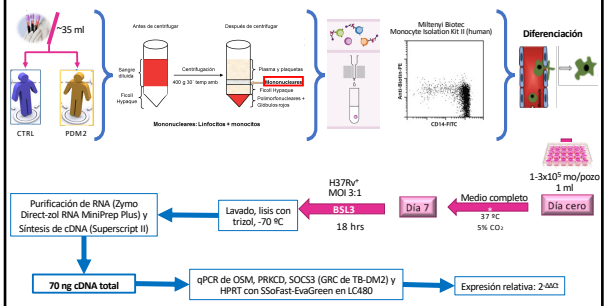
Modelo de MDM infectados con *MTb in vitro* (Etapa 2).



*(RPMI 1640, Suero autólogo 10%, L-glutamina 2uM, Piruvato 1%, β-Mercapto etanol 0.1% y hM-CSF 10 ng/ml).
*En fase exponencial de crecimiento con ADC 10% y Tween-80 0.05%

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La expresión de SOCS3 es menor en MDM de pacientes DM2 con pobre control glicémico tras la infección con *Mtb*

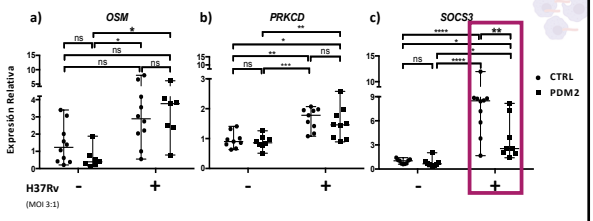

Figura 5. Los monocitos fueron aislados de sangre periférica por selección negativa (CTRL n=10 y PDM2 n=9) y diferenciados a macrófagos (MDM) utilizando M-CSF y suero autólogo. Los MDM fueron infectados (+) o no (-) con H37Rv con una MOI 3:1, durante 18 horas. Se aisló RNA de los MDM y se sintetizó cDNA para utilizar 70 ng por muestra para los ensayos de qPCR. La expresión relativa de a)OSM, b)PRKCD y c)SOCS3 se calculó con la ecuación de $2^{-\Delta\Delta Ct}$ utilizando HPRT como control interno. Se excluyeron las muestras outliers según la prueba de Grubb´s. Las diferencias entre los grupos fueron establecidas utilizando Anova de dos vías y comparaciones múltiples de Bonferroni. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001; con intérvalo de confianza de 95%.

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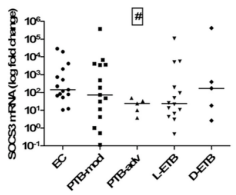
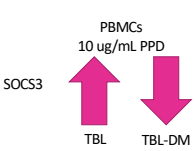
Disminución del RNA de SOCS3 en PBMCs de TB y TBL-DM



Masood, *et al.*, (2013), BMC Infectious Diseases.



Masood, *et al.*, (2021), Scand J Immunol.

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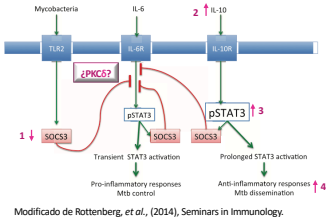
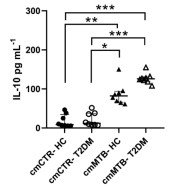
Efecto de la disminución de SOCS3 en MDM



Modificado de Rottenberg, *et al.*, (2014), Seminars in Immunology.



Valtierra-Alvarado M, *et al.*, (2021), ICB.

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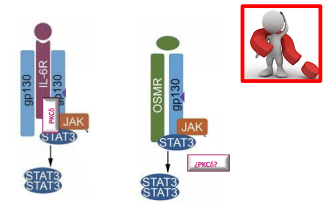
Activavión de STAT3 mediada por PKCδ

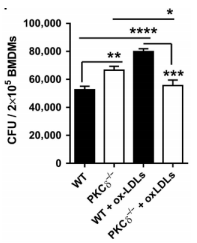
PKCdelta se asocia con gp130 vía STAT3 y potencía la interacción STAT3-gp130

PDM2



Modificado de Novotny-Diermayr V., *et. al.*, (2002), JBC;

Impacto de PKCδ en la eliminación de *Mtb*



SP Parihar, *et. al.*, Mucosal Immunology, 2017.

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Conclusiones

- *OSM*, *PRKCD* y *SOCS3* son Genes Reguladores que se encuentran elevados en su expresión en células de sangre total de sujetos TB-DM2 y en MDM de sujetos con DM2 con pobre control glicémico.
- El análisis de las interacciones entre estos genes reguladores, así como lo observado en las interacciones proteína-proteína indica que hay una expresión alterada y coordinada de estos genes. La alta probabilidad de interacción funcional entre estos genes pudiera explicar la inhibición de los mecanismos metabólicos y de respuesta inmune en MDM de sujetos con el binomio TB-DM2 y DM2 con pobre control glicémico.
- No se pudo demostrar que la expresión de estos Genes Reguladores tuviera un efecto significativo para la eliminación de *Mtb* en MDM de PDM2 *in vitro*.

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Áreas de oportunidad

- Cubrir el objetivo 4 de ensayos de UFC y su asociación con los GRC.
- Validar los niveles de proteína *OSM*, *PRKCD*, *SOCS3* en el modelo in vitro.
- Evaluar la participación de los Genes Reguladores en las vías de señalización *IL6ST*/*STAT3* en MDM de PDM2 infectados in vitro con *Mtb*.
- Validar los Genes Reguladores de DM2 con pobre control glicémico mediante qPCR.
- Demostrar que la interacción entre Genes Reguladores hace más susceptibles a los MDM de DM2 con pobre control glicémico a la infección por Mtb.

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Banco de muestras: UIBMZ-IMSS

- Base de datos únca de TB.
- Base de datos de diabetes varios proyectos.

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Cursos

- "Escribe y publica tu trabajo científico PRO". INMEGEN en línea. Marzo-Abril de 2021.
- "Todo sobre la prevención del COVID-19" IMSS en línea. Noviembre de 2020.
- "ePROTECT Infecciones respiratorias: Salud y seguridad ocupacional" Organización Panamericana de la Salud. Septiembre de 2020.
- "IUIS-ALAI-SMI IMMUNOINFORMATICS COURSE" Centro de Ciencias de la Complejidad (C3), UNAM. Ciudad de México. Abril de 2019.
- "Introduction to R" Certificate number 7,899,395 statement of accomplishment. Data Camp (on line). Marzo de 2019.
- Asistencia al taller "Massive sequencing" del XXVII Foro Nacional de Investigación en Salud, IMSS, Zacatecas, México. Septiembre de 2018.
- Acreditación del modulo práctico 3 "Phenotypic and functional study of NK and dendritic cells (mDC and pDC) in peripheral blood" del II Curso Internacional: inmunidad innata en la salud y las enfermedades infecciosas. Aguascalientes, México. Septiembre de 2018.
- Acreditación del II Curso Internacional: inmunidad innata en la salud y las enfermedades infecciosas. Módulo teórico. Guadalajara, Jalisco, México. Septiembre de 2018.
- Reunión pre-congreso "I Iberoamerican Flow Cytometry Meeting", Cancún, Quintana Roo, México. Mayo de 2018.
- Concurso en la "Exhibición de Carteles de Proyectos de Investigación de Estudiantes Graduados de la UASLP". San Luis Potosí, México. Octubre de 2017.

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Divulgación científica

- "Transcriptional patterns in patients with diabetes under poor glycemic control associated to altered immune functional pathways in patients with tuberculosis-diabetes co-morbidity" en el "17th International Congress of Immunology" Beijing, China. Octubre de 2019.
- "Perfil transcripcional asociado con la co-morbilidad tuberculosis-diabetes en humanos" en el XXVII Foro Nacional de Investigación en Salud, IMSS, Zacatecas, México. Septiembre de 2018.
- "Perfil transcripcional asociado con la co-morbilidad tuberculosis-diabetes en humanos" en el Foro Norte de Investigación en Salud. Durango, Durango. México. Junio de 2018.
- "Transcriptional profile associated with tuberculosis-diabetes co-morbidity in humans" en el XII Congreso de la Asociación Latinoamericana de Inmunología y el XXIII Congreso de la Sociedad Mexicana de Inmunología, Cancún, Quintana Roo, México. Mayo de 2018.

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Colaboraciones

- Oropeza, et. al. “Niveles elevados de arsénico tienen estrecha asociación con la reducción de la función renal en pacientes con diabetes mellitus tipo 2” Galardonado con el 1er Lugar. 53º Congreso Nacional de Nefrología. Zacatecas, México. Septiembre de 2019. Cartel.
- Arias, et. al. Microarreglos de proteínas para la identificación de biomarcadores de diabetes gestacional (2019).
- Abel, et. al., Evaluación de IFN gamma mediante pruebas de QTF Plus (2020) en pacientes con artritis reumatoide.
- Alejandro, et. al., Microarreglos de proteínas de citocinas pro-inflamatorias en suero, después de un tratamiento experimental para mejorar parámetros clínicos de un modelo animal de diabetes (2021).

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Artículos enviados a revistas JCR para proceso de publicación:

Molecular Genetics and Genomics

Potential molecular patterns for tuberculosis susceptibility in diabetic patients with poor glycaemic control: A pilot study

--Manuscript Draft--

Manuscript Number:	
Full Title:	Potential molecular patterns for tuberculosis susceptibility in diabetic patients with poor glycaemic control: A pilot study
Article Type:	Original Article
Corresponding Author:	José Antonio Enciso-Moreno Instituto Mexicano del Seguro Social MEXICO
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Instituto Mexicano del Seguro Social
Corresponding Author's Secondary Institution:	
First Author:	Elena Jaime-Sánchez

Microbes and Infection

Mycobacterium tuberculosis infection is associated with a decreased suppressor of cytokine signaling 3 (SOCS3) expression in macrophages from poorly controlled patients with type 2 diabetes.

--Manuscript Draft--

Manuscript Number:	MICINF-D-22-00266
Article Type:	Short communication
Keywords:	diabetes; gene expression; macrophage; tuberculosis; SOCS3
Corresponding Author:	Carmen Judith Serrano, Ph.D. IMSS: Instituto Mexicano del Seguro Social Zacatecas, Zacatecas MEXICO
First Author:	Elena Jaime-Sánchez, MSc

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

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Publicaciones

> Arch Med Res. 2019 Feb;50(2):71-78. doi: 10.1016/j.arcmed.2019.05.006. Epub 2019 Jun 19.

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

Yamile López-Hernández [1], Edgar E Lara-Ramírez [2], Mariana Salgado-Bustamante [3], Jesús Adrián López [4], Juan J Oropeza-Valdez [2], Elena Jaime-Sánchez [2], Julio E Castañeda-Delgado [5], Martín Magaña-Aquino [6], Michael Mun[illegible] [7], José A Enciso-Moreno [8]

Clinical Trial > PLoS One. 2018 Mar 27;13(3):e0194205. doi: 10.1371/journal.pone.0194205. eCollection 2018.

Transcriptional signature associated with early rheumatoid arthritis and healthy individuals at high risk to develop the disease

N Macías-Segura [1 2], J E Castañeda-Delgado [3], Y Bastian [3], Santiago-Algarra [1 4], J D Castillo-Ortiz [5], A L Alemán-Navarro [1 2], E Jaime-Sánchez [1], M Gomez-Moreno [1 2], C A Saucedo-Toral [1], Edgar E Lara-Ramírez [1], M Zapata-Zuñiga [6], L Enciso-Moreno [1], R González-Amaro [6], C Ramos-Remus [6 7], J A Enciso-Moreno [1]

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