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Differential network analysis between sex of the genes related to comorbidities of type 2 mellitus diabetes

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Abstract

Background: Some phenotypical changes may be related to changes in the associations among genes. The set of such associations is referred to as gene interaction (or association) networks. An association network represents the set of associations among genes in a given condition. Given two experimental conditions, Differential network analysis (DNA) algorithms analyse these differences by deriving a novel network representing the differences. Such algorithms receive as input experimental gene-expression data of two different conditions (e.g. healthy vs. diseased), then they derive experimental networks of associations among genes and, finally, they analyse differences among networks using statistical approaches. We explore the possibility to study possible rewiring due to sex factors, differently from classical approaches.

Methods: We apply DNA methods to evidence possible sex based differences on genes responsible for comorbidities of type 2 diabetes mellitus.

Results: Our analysis evidences the presence of differential networks in tissues that may explain the difference in the insurgence of comorbidities between males and females.

Conclusion: Main contributions of this work are (1) the definition of a novel framework of analysis able to shed light on the differences between males and females; (2) the identification of differential networks related to diabetes comorbidities.

Introduction

The interactions among genes can be modelled using networks (Barabási et al. 2011; Shawn et al. 2022). In such a formalism, nodes represent genes, and the edges represent their associations, both functional and physical ones (Le Novere 2015). The study of these networks may provide relevant information related to the mechanism of cells, such as their changes in different states, e.g. age, sex or diseases (Tang et al. 2011).

Recently, it has been shown that diseases may be characterised not only by gene expression but also by changes in the topology of the networks of the associations among genes (Lichtblau et al. 2017; Ideker and Krogan 2012). For instance, the development and progression of disease cause the rewiring of the gene association

networks; hence the identification of the rewiring patterns may explain at the molecular level the changes related to the diseases (Schadt 2009). In recent years, it has been shown that complex diseases, such as cancer, diabetes, neurodegenerative diseases, and, more recently, COVID-19, exhibit some sex-related differences (Huebschman et al. 2019; Luo et al. 2020; Mercatelli et al. 2021; Tramunt et al. 2020; Mauvais-Jarvis 2015; Succurro et al. 2022; Kim et al. 2018; Whitacre 2001).

We here focus on significant sex differences in the prevalence of type 2 diabetes (T2D) globally, and despite research on this issue, there are some poorly understood mechanisms (Choi et al. 2009; Li et al. 2019). The knowledge of such modifications is the first step towards the design of ad hoc therapeutical strategies.

Recently, *Differential Network Analysis* (DNA) algorithms have been proposed to measure changes in the topology of the networks (e.g. edge rewiring, modification of edge/node weights), representing two different states, such as healthy versus disease (Lichtblau et al. 2017); and DNA algorithms combine the differential expression analysis of genes, i.e., the quantitative difference of the expression level of genes between two states. The network expression analysis (NEA), studies the interplay of the genes on a network level. DNA algorithms are then able to analyse and interpret topological changes in the rewiring in the networks, i.e. changes in the interactors of the genes, and changes in the expression levels (Ideker and Krogan 2012).

We here hypothesise that sex differences in the insurgence, evolution, comorbidities and outcome of the complex disease may be explained in terms of differential networks between males and females. We hence perform a differential network analysis considering as different conditions the sex of the investigated patients (Ideker and Krogan 2012).

We define an innovative pipeline of analysis able to highlight sex-related differences, and we show the effectiveness of such a pipeline in the study of comorbidities of type 2 diabetes mellitus (Peters and Woodward 2018; Meisinger et al. 2002).

T2DM is a complex metabolic disorder characterised by a progressive loss of b-cell insulin secretion, causing hyperglycemia against a background of insulin resistance (Iglay et al. 2016; Succurro et al. 2022; Bellary et al. 2021; Pirillo et al. 2021; Succurro et al. 2023). Demographic analysis of T2DM incidence revealed that comorbidities increase with age and in males (Pearson-Stuttard et al. 2022; Guerrero-Fernández de Alba et al. 2020; Dworzynski et al. 2020; Nowakowska et al. 2019; Menke et al. 2015).

We consider gene expression level data of the genes related to type 2 diabetes comorbidities downloaded from the GTEx database (Lonsdale et al. 2013). We subdivide them into male and female datasets, and we consider the pattern of progression with age in males and females. We apply the hierarchical differential network analysis presented in Lichtblau et al. (2017). This method can identify the differential expression of individual genes when identifying differential edges, and it outperforms the current state-of-the-art methods (Jia-Juan et al. 2021) such as (Bien et al. 2015; Zhang et al. 2019; Yuan et al. 2017). The pipeline we implemented is depicted in Fig. 1.

We found differential networks for six different tissues (Adipose Subcutaneous, Adipose Visceral, Hearth, Liver, Pancreas and Spleen). The functional analysis of these networks revealed some interesting mechanisms that are also validated in the literature.

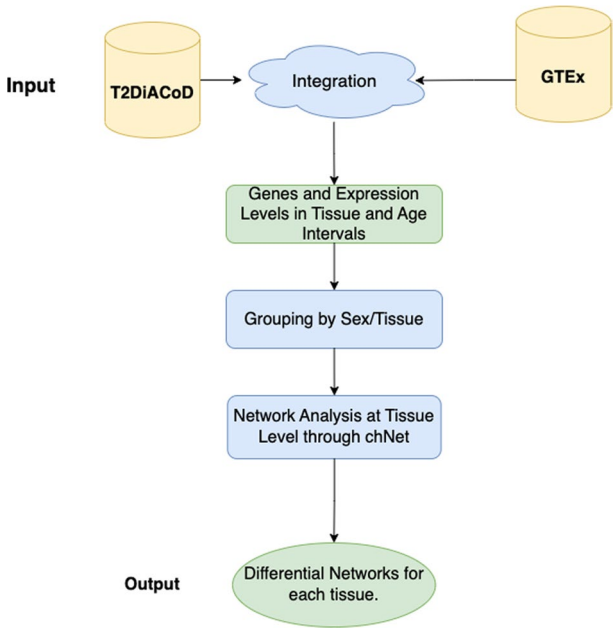

Fig. 1 Workflow of the experiment

Methods

DNA algorithms

DNA algorithms aim to identify changes in the network structures between two states, or conditions (Shojaie 2021). In biology, DNA algorithms have been used to identify changes between the healthy and diseased status of the same biological system (Grimes et al. 2019). There exist some different formulations of the problem, we here focus on networks with the same node sets and different edge sets. Formally, given two different conditions $\mathcal{C}_1$, and $\mathcal{C}_2$, represented by means of two graphs $G_1(V, E_1)$ and $G_1(V, E_2)$, DNA aims to identify changes between them.

When dealing with biological systems it should be noted, that nodes are directly measurable, while edges among them should be derived from a set of observations over time. For instance, when considering gene networks derived from micro-array experiments, nodes are fixed while edges should be inferred from the observations by means of *statistical graphical models* (Lauritzen Lauritzen; Roy et al. 2018; Galicia et al. 2020). In a statistical graphical model, we use a graph $G = (V, E)$, and each node $v \in V$ is associated with a set of m random variables $X_1, \ldots, X_m$ representing quantitative measurements of v, and edges are inferred from $X_1, \ldots, X_m$. We focus on undirected graphs.

From the existing ones, we choose hierarCHical differential NETwork analysis model (chNet) to estimate differential networks since it is able to infer hierarchical properties of the networks. The authors define a differential network as the difference of partial correlations between two conditions. Changes are measured by means of ad hoc developed test statistics to evaluate modifications in the partial correlation between gene pairs. Moreover, the changes in gene expression levels are quantified by using the classical Student's t-test statistics (Zimmerman 1987).

Finally, the two test statistics are integrated into a single optimisation model which aims to evidence the hierarchical structures of networks.

Databases

The list of genes related to diabetes comorbidities were extracted from the T2DiACoD database (Rani et al. 2017). Gene expression data were downloaded from GTEx database (Lonsdale et al. 2013).

The T2DiACoD database stores a curated collection of genes and non-coding RNAs which are related to T2DM. The database has been populated by manual collection and curation of data extracted from literature and existing databases. The authors focused on comorbidities such as atherosclerosis, nephropathy, diabetic retinopathy, and cardiovascular diseases. It actually lists 650 genes and 34 microRNAs related to comorbidities.

The Genotype-Tissue Expression (GTEx) data portal is a publicly available resource which collects genomic data of individuals. Genomic data span a large class of types: from sequencing to methylation.

For each sample, GTEx provides metadata regarding patients, such as tissue of provenance, sex and age (grouped into six classes). Thus GTEx is a primary data source for age-tissue-dependent studies. The current version of the GTEx database accessed on February 01st stores 17,382 samples of 54 tissues of 948 donors, see at https://gtexportal.org/home/tissueSummaryPage. It is available through a web interface offering easy and efficient data querying and visualization (Stanfill and Cao 2021; Mercatelli et al. 2021; Pressler et al. 2022; Ortuso et al. 2021). Data may also be downloaded and analysed through an ad hoc realised script. In this study, we used data from different tissues. We build two classes considering the sex. For each tissue, we randomly selected the same number of samples. Since each sample in GTEx has associated with the age of the donor, we also equally balanced age subgroups into each group. Thus, for each tissue, we have the same number of samples, and the age distribution is homogeneous.

Differential network analysis was performed by using the chNet package (Jia-Juan et al. 2021), which is currently a state-of-the-art method. chNet was originally designed for performing differential network analysis between two conditions. Here we applied the package in an innovative way to compare males versus females. chNet implements a differential network analysis method which considers both differential expressions of genes and differential edges among conditions. It initially tests changes in partial correlations between gene pairs and changes in expression levels for individual genes. Then it combines these results to build hierarchical networks where a differential edge can be considered only if at least one of the two involved genes is differentially expressed. The chNet package was used to analyze 23 tissues, and several runs were made for each by varying the lambda adjustment parameter, which controls the sparsity level of the obtained graph. There is no one-size-fits-all choice of lambda, as evidenced in Jia-Juan et al. (2021), thus we need to test many values of lambda. We considered many values of lambda (2; 3; 4; 8; 10; 12) using a heuristic approach and we finally selected the value 12. Higher values of lambda produced no significantly enriched networks.

Table 1 Characteristics of the differential networks

Organ	Nodes	Edges
Adipose visceral	343	508
Adipose subcutaneous	246	609
Hearth	382	777
Liver	347	1259
Pancreas	346	358
Spleen	420	1534

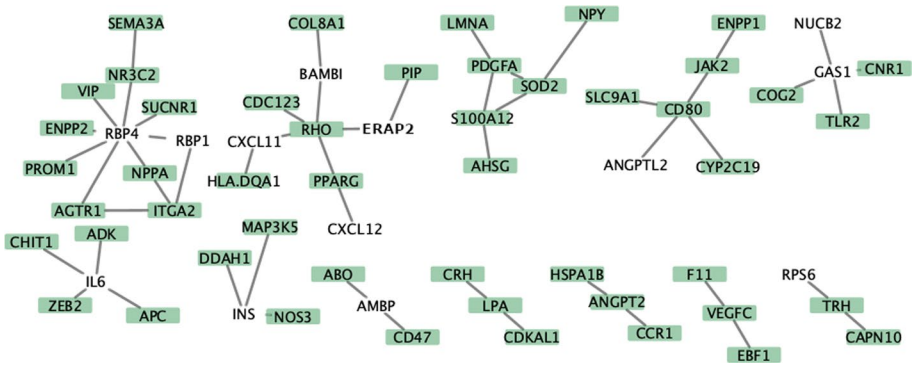

Fig. 2 Differential network for subcutaneous adipose tissue. Differentially expressed nodes are highlighted in green. For better visualization, we clustered the network using Markov Clustering and plotted only the largest clusters

Results

Identifying the rewiring of gene networks between different sex may give insights into the molecular mechanisms explaining different incidences and prognoses of comorbidities of T2DM. We aim to estimate differential networks between males and females using gene expression datasets from GTEx database for Adipose Visceral, Adipose Subcutaneous, Hearth, Liver, Pancreas and Spleen. Table 1 summarizes the characteristics of the obtained networks. For each tissue we also evidence differentially expressed nodes in green. The hierarchical constraints of chNET ensure that every differential edge is incident to at least one differentially expressed gene while two non-differentially expressed genes cannot be linked.

For each network we analysed the pathway enrichment considering the KEGG (Kanehisa 2002) pathway database by using the STRING enrichmennt app of the Cytoscape software (Doncheva et al. 2018). Figures 2, 3, 4, 5, 6, 7, 8, and 9 depict some selected subnetworks representing biggest networks obtained by clustering with Markov Clustering the networks of chNet package. In each figure differentially expressed genes are highlighted in green. We also present in the following Tables 2, 3, 4, 5, 6, and 7, the main enriched pathways of the differential networks for each tissue.

Subcutaneous adipose tissue

The adipose tissue subcutaneous differential network has 246 nodes and 609 edges. Figure 2 depicts some subnetworks. Pathway analysis reveals the presence of some enriched pathways between sex.

Table 2 Pathway analysis of adipose subcutaneous tissue

Term name	Description	FDR value
hsa04151	PI3K-Akt signaling pathway	1.57E−13
hsa04068	FoxO signaling pathway	3.76E−10
hsa05200	Pathways in cancer	7.15E−10
hsa04080	Neuroactive ligand-receptor interaction	1.03E−9
hsa04066	HIF-1 signaling pathway	1.62E−8
hsa04668	TNF signaling pathway	2.6E−8
hsa04061	Viral protein interaction with cytokine and cytokine receptor	3.89E−8
hsa05205	Proteoglycans in cancer	4.8E−8
hsa04931	Insulin resistance	1.01E−7
hsa05418	Fluid shear stress and atherosclerosis	1.01E−7
hsa04920	Adipocytokine signaling pathway	1.1E−7
hsa05206	MicroRNAs in cancer	1.18E−7
hsa05163	Human cytomegalovirus infection	1.32E−7
hsa04933	AGE-RAGE signaling pathway in diabetic complications	2.47E−7
hsa01521	EGFR tyrosine kinase inhibitor resistance	2.55E−7
hsa04015	Rap1 signaling pathway	2.55E−7

All the pathways have False Discovery Rate of less than 0.01

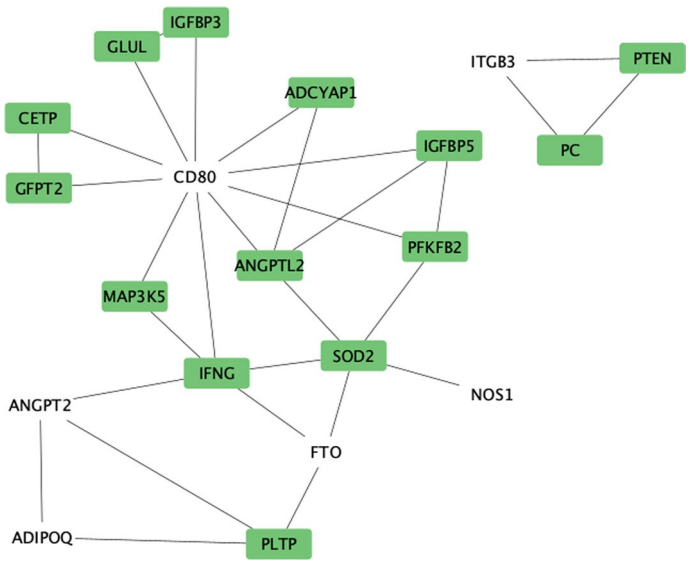

Fig. 3 Differential network for adipose visceral tissue. differentially expressed nodes are highlighted in green. For better visualization, we clustered the network using Markov Clustering and plotted only the largest clusters

Visceral adipose tissue

Differential network of the adipose tissue Visceral has 343 nodes and 508 edges. Figure 3 depicts some subnetworks. Pathway analysis reveals the presence of some enriched pathways between sex.

Table 3 Pathway analysis of adipose visceral tissue

Term name	Description	FDR value
hsa00071	Fatty acid degradation	2.22E−11
hsa04022	cGMP-PKG signaling pathway	2.22E−11
hsa00010	Glycolysis/gluconeogenesis	2.03E−8
hsa04923	Regulation of lipolysis in adipocytes	2.81E−8
hsa04261	Adrenergic signaling in cardiomyocytes	5.17E−8
hsa04270	Vascular smooth muscle contraction	8.29E−8
hsa00410	Beta-Alanine metabolism	2.37E−7
hsa00280	Valine, leucine and isoleucine degradation	5.84E−6
hsa00340	Histidine metabolism	5.98E−6
hsa04979	Cholesterol metabolism	6.7E−6
hsa04927	Cortisol synthesis and secretion	6.83E−6
hsa00230	Purine metabolism	7.91E−6
hsa04024	cAMP signaling pathway	1.17E−5
hsa00380	Tryptophan metabolism	1.72E−5
hsa04020	Calcium signaling pathway	2.04E−5
hsa04213	Longevity regulating pathway—multiple species	1.4E−4
hsa03320	PPAR signaling pathway	3.8E−4

All the pathways have False Discovery Rate of less than 0.01

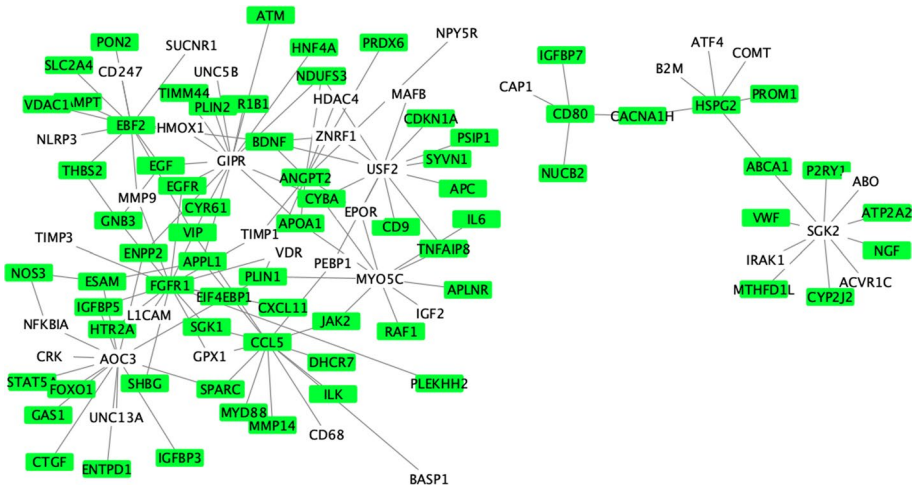

Fig. 4 A Selected subnetwork of the differential network in Hearth Tissue. For better visualization, we clustered the network using Markov Clustering and plotted the largest clusters. Differentially expressed nodes (genes) are highlighted in green

Hearth

The differential network of the hearth has 382 nodes and 777 edges. Figure 4 depicts some subnetworks. Pathway analysis reveals the presence of some enriched pathways between sex.

Table 4 Pathway analysis of hearth tissue

Term name	Description	FDR value
hsa00010	Glycolysis/gluconeogenesis	6.91E−12
hsa00071	Fatty acid degradation	6.91E−12
hsa04022	cGMP-PKG signaling pathway	6.78E−9
hsa00350	Tyrosine metabolism	2.25E−8
hsa04923	Regulation of lipolysis in adipocytes	6.55E−8
hsa04261	Adrenergic signaling in cardiomyocytes	1.98E−7
hsa04270	Vascular smooth muscle contraction	3.03E−7
hsa00340	Histidine metabolism	8.16E−7
hsa00380	Tryptophan metabolism	5.07E−6
hsa00410	Beta-Alanine metabolism	5.1E−6
hsa05204	Chemical carcinogenesis	7.34E−6
hsa04020	Calcium signaling pathway	1.97E−5
hsa00620	Pyruvate metabolism	2.33E−5
hsa01230	Biosynthesis of amino acids	3.49E−5
hsa02010	ABC transporters	0.0017
hsa03320	PPAR signaling pathway	0.0134
hsa04911	Insulin secretion	0.0187

All the pathways have a False Discovery Rate of less than 0.01

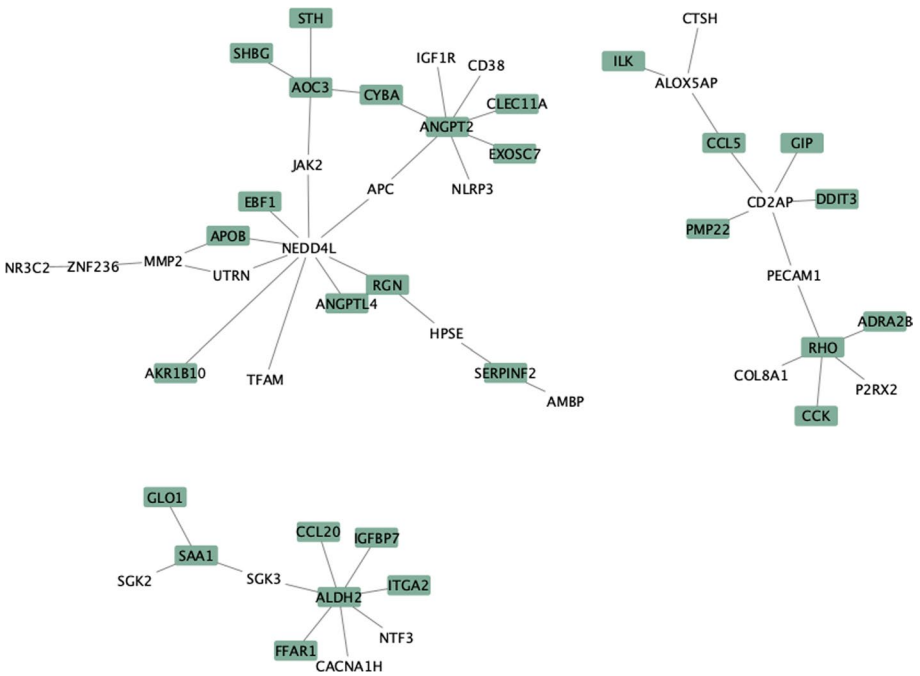

Fig. 5 A Selected subnetwork of the differential network in Liver Tissue. For better visualization, we clustered the network using Markov Clustering and plotted the largest cluster. Differentially expressed nodes (genes) are highlighted in green

Liver tissue

The differential network of the livre has 347 nodes and 1259 edges. Figures 5 and 6 depict some subnetworks (we split the subnetwork into two figures for

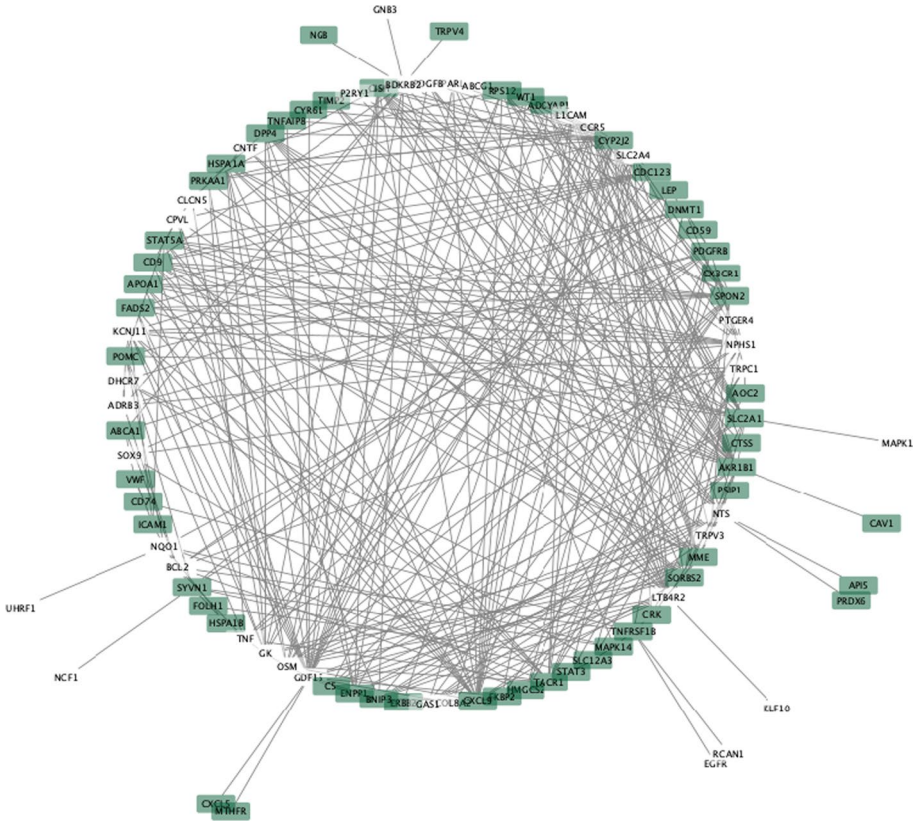

Fig. 6 A Selected subnetwork of the differential network in Liver Tissue. For a better visualization, we clustered the network by using Markov Clustering and we plot here the biggest cluster. Differentially expressed nodes (genes) are highlighted in green

Table 5 Pathway analysis of liver subcutaneous tissue

Term name	Description	FDR value
hsa01100	Metabolic pathways	2.96E−16
hsa00071	Fatty acid degradation	1.82E−12
hsa00010	Glycolysis/gluconeogenesis	2.62E−10
hsa04022	cGMP-PKG signaling pathway	1.32E−9
hsa00350	Tyrosine metabolism	7.99E−9
hsa04970	Salivary secretion	7.99E−9
hsa04270	Vascular smooth muscle contraction	1.19E−7
hsa04923	Regulation of lipolysis in adipocytes	2.74E−7
hsa04261	Adrenergic signaling in cardiomyocytes	3.41E−7
hsa00830	Retinol metabolism	1.04E−6
hsa00380	Tryptophan metabolism	2.77E−6
hsa05204	Chemical carcinogenesis	3.69E−6
hsa00280	Valine, leucine and isoleucine degradation	5.55E−6
hsa00982	Drug metabolism—cytochrome P450	7.01E−6
hsa04020	Calcium signaling pathway	7.01E−6
hsa00410	Beta-Alanine metabolism	3.07E−5

All the pathways have a False Discovery Rate of less than 0.01

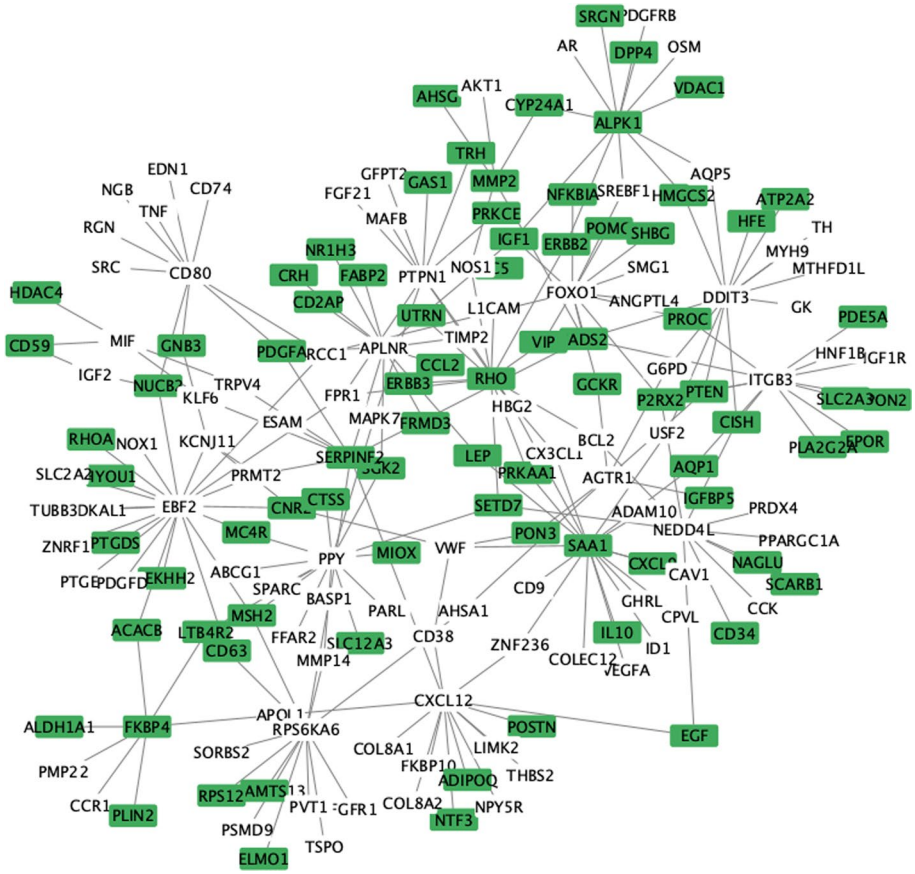

Fig. 7 A Selected subnetwork of the differential network in Pancreas Tissue. For a better visualization, we clustered the network by using Markov Clustering and we plot here the biggest cluster. Differentially expressed nodes (genes) are highlighted in green

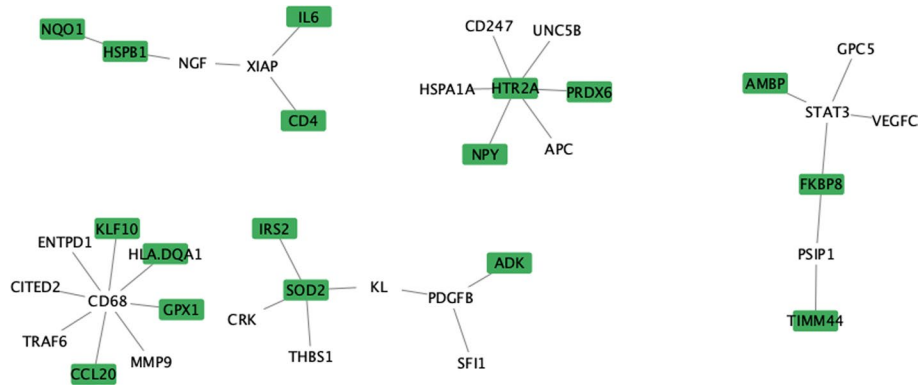

Fig. 8 For better visualization, we clustered the network by using Markov Clustering and plotted only the largest clusters

better readability). Pathway analysis reveals the presence of some enriched pathways between sex.

Table 6 Pathway analysis of pancreas tissue

Term name	Description	FDR value
hsa04151	PI3K-Akt signaling pathway	8.45E−20
hsa05200	Pathways in cancer	1.16E−18
hsa05418	Fluid shear stress and atherosclerosis	1.97E−17
hsa05206	MicroRNAs in cancer	1.67E−16
hsa04015	Rap1 signaling pathway	3.74E−15
hsa04068	FoxO signaling pathway	7.0E−15
hsa04010	MAPK signaling pathway	7.58E−15
hsa05205	Proteoglycans in cancer	9.21E−15
hsa01521	EGFR tyrosine kinase inhibitor resistance	1.09E−14
hsa04510	Focal adhesion	7.24E−14
hsa05215	Prostate cancer	2.77E−13
hsa05163	Human cytomegalovirus infection	5.07E−13
hsa04931	Insulin resistance	1.42E−12
hsa04933	AGE-RAGE signaling pathway in diabetic complications	3.2E−12
hsa04080	Neuroactive ligand-receptor interaction	4.24E−12
hsa04668	TNF signaling pathway	2.29E−11
hsa04211	Longevity regulating pathway	4.4E−10

All the pathways have a False Discovery Rate of less than 0.01

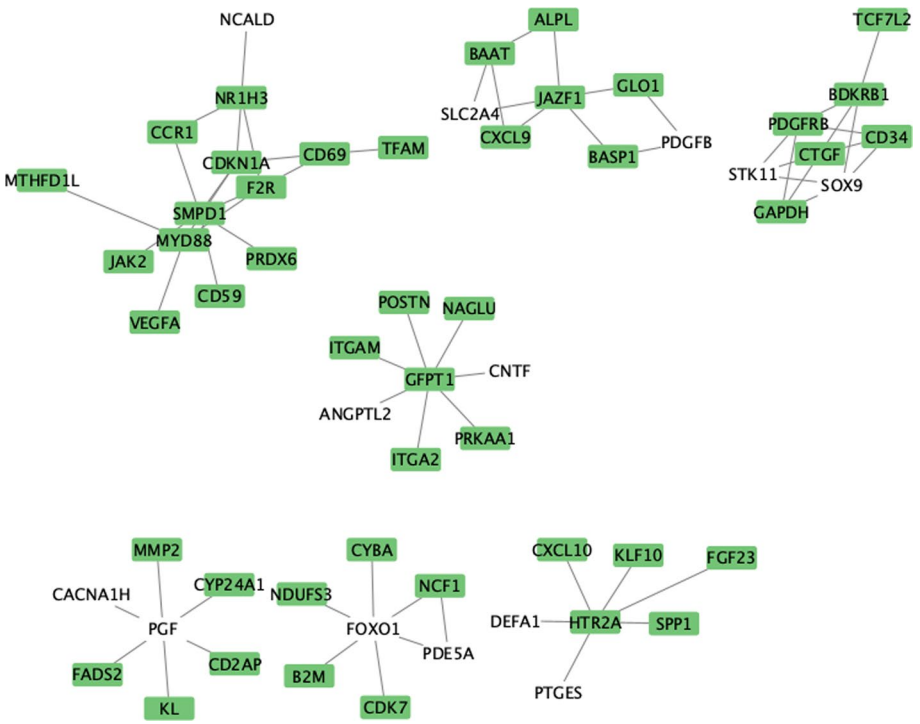

Fig. 9 Some selected subnetworks of the differential network considering the expression of genes in Spleen tissue. For better visualization, we clustered the network using Markov Clustering and plotted only the largest clusters

Pancreas tissue

The differential network of the liver has 346 nodes and 358 edges. Figures 7 and 8 depict some subnetworks (we split the subnetwork in two figures for better readability).

Table 7 Pathway analysis of spleen subcutaneous tissue

Term name	Description	FDR value
hsa05200	Pathways in cancer	2.04E−23
hsa04151	PI3K-Akt signaling pathway	2.87E−23
hsa05418	Fluid shear stress and atherosclerosis	3.1E−21
hsa01521	EGFR tyrosine kinase inhibitor resistance	4.72E−17
hsa04510	Focal adhesion	1.25E−16
hsa04010	MAPK signaling pathway	3.21E−16
hsa04068	FoxO signaling pathway	6.03E−16
hsa04015	Rap1 signaling pathway	1.03E−15
hsa04933	AGE-RAGE signaling pathway in diabetic complications	1.99E−15
hsa04668	TNF signaling pathway	2.64E−15
hsa05215	Prostate cancer	9.63E−15
hsa05145	Toxoplasmosis	4.8E−14
hsa04066	HIF-1 signaling pathway	5.38E−14
hsa04211	Longevity regulating pathway	6.4E−11
hsa04014	Ras signaling pathway	2.14E−10
hsa04061	Viral protein interaction with cytokine and cytokine receptor	2.38E−10
hsa04910	Insulin signaling pathway	8.87E−8

All the pathways have a False Discovery Rate of less than 0.01

Pathway analysis reveals the presence of some enriched pathways between sex.

Spleen tissue

The differential network of the Spleen has 420 nodes and 1534 edges. Figures 5 and 6 depict some subnetworks (we split the subnetwork into two figures for better readability). Pathway analysis reveals the presence of some enriched pathways between sex.

Discussion

In this paper, we started from the observation that the incidence of comorbidities in T2DM presents a different behaviour in males and females (Succurro et al. 2022; Roche and Wang 2013). Then, we searched for possible sex-based differences at the molecular level of the genes related to such comorbidities. To highlight this mechanism, we hypothesised the existence of a differential network between males and females. Then we searched for these differential networks by using chNet (Jia-Juan et al. 2021). The first contribution of our work is showing the existence of these differential networks discriminating between sex. Since there is no true differential network between the investigated states, we cannot compare our networks to a reference benchmark. Therefore, we analyse the functional significance of the obtained networks. The functional analysis we made shows some interesting pathways. First, we found in Adipose Tissue Subcutaneous and Pancreas a modulation of genes related to PI3K/AKT pathway activity (see Tables 2 and 6). This pathway is related to insulin resistance mechanism, and obesity and modifications leading to diabetes occur in both Pancreas and adipose tissue (Huang et al. 2018). We here extend these results by evidencing that these modifications are different in males and females, thus suggesting sex differences in insulin resistance mechanism as already investigated in Ortiz-Huidobro et al. (2021).

While the PI3K/Akt signalling pathway regulates the primary metabolic functions of the insulin, the MAPK signalling pathway regulates the mitogenic effects of insulin (Sidarala and Kowluru 2016). Pathway analysis we performed also evidenced the presence of this pathway in differential networks of the Pancreas and Spleen (see Tables 6, and 7).

In Regitz-Zagrosek and Kararigas (2017); Foryst-Ludwig et al. (2011) the authors showed increased physiological myocardial hypertrophy in females.

Not surprisingly, adipose tissue (both subcutaneous and visceral) present many enriched pathways, thus confirming sexual differences at the molecular level as previously indicated in Fuente-Martín et al. (2013); Bond et al. (2021).

We also found in the Visceral Adipose Tissue the modification of cAMP signalling pathway (Jeremiah Ong'achwa et al. 2019). cAMP-dependent pathway mediates many cellular responses, controls the increase in heart rate and the level of cortisol secretion (that we found also modulated as shown in Table 3), and breakdown of glycogen and fat. Tengholm and Gylfe (2017). Moreover, it plays a central role in regulating insulin and glucagon secretion from the pancreatic cells and acts as an insulin secretion amplifier. The dysregulation of this pathway is generally associated with diabetes (Tengholm and Gylfe 2017). Our findings show that there are also differences at sex level. Thus, therapeutic interventions for normalising insulin and glucagon secretion should be tailored considering sex.

We should note that in Pancreas tissue, we found the enrichment of the AGE-RAGE signalling pathway (Litwinoff et al. 2015) (see Table 6). This result is interesting for two main reasons. First, this pathway is involved in the pathogenesis of both micro, and macrovascular complications of diabetes (Litwinoff et al. 2015). Second, the sex-specific mechanism of RAGE deficiency in the regulation of adipose tissue and the homeostasis of glucose has been recently demonstrated (Zuoqin et al. 2022), corroborating our finding. Similarly, we found that FoxO signalling pathway is enriched in the differential network of Pancreas tissue (Yoshihara et al. 2019). FoxO pathway has an essential role in developing diabetic kidney disease (Kato et al. 2006). Recently, in Tower et al. (2020) the authors discussed sex differences in the stress response of cells and tissues, evidencing that female cells are generally more resistant to stress-induced cell death.

We found that differential networks in Adipose Visceral Tissue and Hearth (see Tables 3, ad and 4) are also enriched for cGMP-PKG signalling pathway (Frigolet et al. 2017; Xue et al. 2019). This pathway mediates the inhibition of the adverse regulatory effects of MAPK/JNK and is related to the augmentation of insulin signalling and glucose uptake via modulation of JNK activity, which is dependent on the sGC-cGMP-PKG pathway. The dysregulation of this pathway is associated with heart failure (Numata and Takimoto 2022). However, our findings suggest that our studies are needed to understand these sex-related differences for the optimal therapeutic strategy with the enhancement of cGMP-PKG signalling pathway. Moreover, the enrichment of the PPAR signaling pathway we found confirms other previous studies such as Park and Choi (2017) Benz et al. (2012), thus corroborating the method and the results we found.

As a final remark, we may affirm that this study evidences the existence of differential networks of the genes related to T2DM comorbidities based on the sex of the individuals. So, we may generate the hypothesis that more studies should be done to confirm this

hypothesis in population, also integrating multi-omics data in a multiscale perspective (Shawn et al. 2022; Cho et al. 2013; Gallo Cantafio et al. 2018).

Conclusion

We focused on differential networks at tissue level which may explain phenotipical differences in diabetes comorbidities insurgence and progression. There is clinical and epidemiological evidence of different establishment and progression of T2DM comorbidities in males and females. We found some differential networks that may help to shed out light on the molecular causes of these differences. Moreover, the elucidation of these mechanisms may help in future to design sex-targeted therapeutic strategies as well as to suggest tailored public health policies.

Author contributions
PHG and EP conceived the main ideas of the paper. PHG and PV supervised bioinformatics analysis. FC wrote the code and performed the bioinformatics analysis. GCM, ES, and FA participated in the validation of biological results. GCM and FA supervised the biological aspects of the paper. PHG and PV wrote the manuscript. All the authors have read and approved the manuscript.

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Availability of data and materials
All the datasets underlying this article are publicly available. Data and source code can be accessed through the web at https://github.com/hguzzi/DifferentialNetworkDiabetes.

Declarations

Ethics approval and consent to participate
Not applicable.

Competing interests
The authors declare that they do not have competing interests.

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