Highly perturbed genes and hub genes associated with type 2 diabetes in different tissues of adult humans: A bioinformatics analytic workflow

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ABSTRACT

Introduction: Type 2 diabetes (T2D) has a complex etiology which is not fully elucidated.

Identification of gene perturbations and hub genes of T2D may assist in personalizing care.

Objectives: We aimed to identify highly perturbed genes and hub genes associated with T2D in different tissues of adult humans via an extensive workflow.

Methods: Workflow comprised five sequential steps: systematic review of NCBI GEO database; identification and classification of differentially expressed genes (DEG); identification of highly perturbed genes via meta-analysis; identification of hub genes via network analysis; downstream analyses. Three meta-analytic strategies: random effects model (REM); vote counting approach (VC); *p*-value combining approach (CA), were applied. Nodes having above average betweenness, closeness, and degree in the network were defined as hub genes. Downstream analyses included gene ontologies, Kyoto Encyclopedia of Genes and Genomes pathways, metabolomics, COVID-19 related genes, and Genotype-Tissue Expression profiles.

Results: Analysis of 27 eligible microarrays identified 6284 DEG (4592 down-regulated and 1692 up-regulated) within four tissue types. Tissue-specific gene expression was significantly greater than tissue non-specific (shared) gene expression. Meta-analysis of DEG identified 49, 27, and 8 highly perturbed genes via REM, VC, and CA, respectively, producing a compiled set of 79 highly perturbed (41 down-regulated and 38 up-regulated) genes. The 28 hub genes comprised 13 up-regulated, 9 down-regulated, and 6 predicted genes. Downstream analyses identified enrichments of: shared genes with other diabetes phenotypes; insulin synthesis and action related pathways and metabolomics; mechanistic associations with apoptosis and immunity-related pathways, COVID-19 related gene sets; and cell types demonstrating over- and under-expression of marker genes of T2D.

Conclusions: We identified highly perturbed genes and hub genes of T2D and revealed their associations with other diabetes phenotypes and COVID-19 as well as pathophysiological manifestations such as those related to insulin, immunity, and apoptosis. Broader utility of the proposed pipeline is envisaged.

Keywords: Type 2 diabetes; Highly perturbed genes; Hub genes; Meta-analysis; Differential gene expression; COVID-19

1. INTRODUCTION

According to an analysis of global data through years 1990-2018, diabetes was prevalent in almost half a billion people, a number expected to rise by 25% and 51%, respectively, by 2030 and 2045 [1]. Type 2 diabetes (T2D) is by far the most common, accounting for over 90% of all diabetes cases [2], a condition found to have affected 6.3% (462 million) of the world population as in year 2017 [3]. Current evidence suggests a complex and multifactorial etiology of T2D characterized by genetic and environmental interactions [4], although T2D pathogenesis is not yet fully elucidated. Moreover, there is evidence of varying degrees of shared genetic origins in the pathogenesis of T2D and other diabetes phenotypes such as type 1 diabetes (T1D) [5], latent autoimmune diabetes in adults (LADA) [6], and maturity-onset diabetes of the young (MODY) [7]. Recent studies also support the deconstruction of T2D heterogeneity to define T2D sub-types [8] and the delineation of a continuum of diabetes sub-types [9] as opposed to the status quo characterized by a few distinct diabetes phenotypes. Understanding the genetic basis of T2D is fundamental to precision medicine approaches striving for impeccable matching and an individualized level of T2D care [10].

Hyperglycemia in T2D is triggered by both impaired action and secretion of insulin [11], unlike T1D the hallmark of which is a lack of insulin synthesis owing to autoimmune-mediated pancreatic β -cell destruction [12]. Cardinal tissues of the body impacted by heightened insulin resistance (IR) and diminished insulin secretion in T2D include the pancreas, liver, skeletal muscle, and adipose tissue [13]. Alterations in the pancreas with T2D include the deposition of β -amyloid [14], reduced β -cell mass and increased β -cell apoptosis [15], as well as decreased size, irregularities of morphology and increased fat content [16]. A salient manifestation of T2D is the compromised insulin-mediated glucose uptake predominantly by skeletal muscle and to a lesser extent by adipose tissue resulting in hyperglycemia [17]. More granular findings associated with T2D such as modifications of

skeletal muscle proteins responsible for work capacity [18] and reduction of skeletal muscle capillary density and microvascular function [19] have also been reported. Adipose tissue inflammation [20] and lipotoxicity [21] have been implicated in T2D pathogenesis. These adipose tissue changes in people with T2D may be caused by numerous transcriptional and epigenetic changes [22]. Similarly, liver fat has been implicated in the pathogenesis of T2D [23] possibly via oxidized fatty acids [24].

Exploration of gene perturbation in T2D can deepen our understanding of the molecular etio-pathology of T2D. Highly up- and down-regulated genes expressed consistently across different tissue types may uncover potential genome-wide biomarkers of the disease. Identification of such gene signatures is also an integral component of precision diagnostic, prognostic, monitoring, and treatment approaches. Topologically, hub genes are defined as highly and tightly connected nodes in typically scale-free gene regulatory networks (GRN) [25]. As such, criteria such as high correlation in candidate modules [26] and above-average betweenness, closeness, and degree [27] in GRN have been used to demarcate hub genes in previous studies. Functionally, they perform critical regulatory roles in biological processes interacting with many other genes in associated pathways. Given their crucial structural and functional characteristics, hub genes are highly sought-after in precision medicine approaches as plausible niches for developing drug and treatment targets [28]. In this context, the importance of identifying highly perturbed genes and hub genes associated with T2D for the purpose of individualizing T2D care is unequivocal.

Downstream analyses of gene sets provide invaluable insights into associated core biological functions, pathways, diseases, drugs and many other aspects. Frequently used gene ontology (GO) analysis provide evidence as a snapshot of contemporary biological knowledge related to a given gene including its function at the molecular level, the cellular location(s) it functions at and the biological processes reliant on it [29]. Pathway analyses such as Kyoto

Encyclopedia of Genes and Genomes (KEGG) [30] entail derivation of coherent and meaningful biological phenomena attributable to input genes [31]. Besides classic GO and pathway analyses, further downstream analyses such as diseases, drugs, metabolomes, and tissue enrichment are also available and can provide valuable insights into associated genes. Taken together, downstream analysis of highly perturbed- and hub genes associated with T2D may render valuable information on aspects such as affected biological processes, dysregulated pathways, related diseases, and metabolomic biomarkers.

Advances in high-throughput technologies have generated a wealth of gene expression data whilst the availability of open-source platforms such as the National Center for Biotechnology Information Gene Expression Omnibus (NCBI GEO) [32, 33] and simultaneous advent in big data and bioinformatics analytic tools such as microarray and RNA-seq meta-analysis and gene-gene interaction network analysis strategies have offered unprecedented opportunities for high-level evidence synthesis from a multitude of gene expression datasets. Such approaches are likely to render new knowledge on complex diseases like T2D and acquire adequate statistical power to identify genes associated with a disease that may not have been evoked via prior analysis of a single or a few datasets.

Yet, to date, no comprehensive evidence synthesis study has been performed to identify highly perturbed genes and hub genes associated with T2D in human adults using an extensive bioinformatics analytic pipeline. Prior studies were limited to identifying hub genes in a few ($n = 3$) microarrays from a single tissue type (pancreatic islets) [34] or performing an *ad hoc* gene expression meta-analysis of all diabetes phenotypes ($n = 13$) [35]. In this study, we aimed to identify highly perturbed genes and hub genes associated with T2D in different tissues of adult humans via a pre-defined and extensive bioinformatics analytic workflow consisting of systematic review, meta-analysis, identification and classification of differentially expressed genes (DEG), network analysis and downstream analysis.

2. METHODS

The methodological approach is summarized in **S1 Figure** and consisted of five sequential steps: (1) Systematic review of NCBI GEO expression data and related publications (2) Analysis of microarrays to identify DEG (3) Meta-analysis of DEG to identify highly perturbed genes in T2D (4) Network analysis of gene-gene/protein-protein interactions to identify hub genes in T2D (5) Downstream analysis of highly perturbed genes and hub genes associated with T2D.

2.1. Systematic review

A preliminary search on the NCBI GEO database was first run on 1st June 2021 using a pre-defined search string: “(“diabetes mellitus, type 2”[MeSH Terms] OR type 2 diabetes[All Fields]) AND “Homo sapiens”[porgn] AND “Expression profiling by array”[Filter]”. The resulting 178 microarrays and related publications were further screened against pre-defined eligibility criteria. Microarrays of conditions other than T2D, all diabetes phenotypes other than T2D, as well as early dysglycemic conditions such as impaired glucose tolerance (IGT), insulin resistance (IR), and prediabetes were excluded. Studies with non-human specimens, children, without healthy controls or with notable co-morbidity in control samples were also excluded. We also omitted studies involving long non-coding RNA (lncRNA), micro RNA (miRNA), samples subject to drug treatments and other interventions, pluripotent stem cells, xenografts, transfected or transgenic tissues, undifferentiated tissues, and sub-samples in super-series. This resulted in 45 microarrays for manual curation which were further screened along with the full texts of related publications (where available) against eligibility criteria and for the presence of adequate information such as clinical diagnosis (healthy vs T2D), gene symbol/Entrez ID. Following this, 27 microarrays were selected for subsequent analyses, the details of which are provided in **S2 Table**.

2.2. Identification of DEG

The 27 microarrays were imported to *R* using ‘*getGEO*’ function of ‘*GEOquery*’ package [36]. In each expression set, ‘*phenoData*’ component was examined to determine the number of eligible samples and confirm the presence of outcome (T2D vs controls) variable whilst ‘*featureData*’ component was explored to verify the presence of gene annotation information. Where required, non-Normalized gene expression matrices were $\log_2$ transformed in order to alleviate skewness and create symmetric distributions [37]. Samples were assessed for the presence of any batch effects between the two groups by running principal components analysis on transposed expression matrices and were rectified using ‘*removeBatchEffect*’ function in ‘*limma*’ package [38]. Relevant features were annotated with expression matrices to generate curated data for running DEG analysis. Samples were ascribed to the relevant group (T2D/control) using ‘*model.matrix*’ function of ‘*limma*’ package [38] producing a binary design matrix. As the detection of DEG can be enhanced by filtering genes with a low expression level, we assumed a median (50%) cut-off for the gene expression level. A uniform analytic pipeline consisting of the following sequential steps were applied to each of the 27 microarrays: (1) Median expression levels were calculated and those above the median were retained. (2) From the resulting genes, those expressed in more than two samples were retained while the others were removed. (3) Model fitting was performed using ‘*lmFit*’ function of ‘*limma*’ [38] to enumerate expression levels of T2D and control groups. (4) Contrasts were defined as ‘T2D – control’ and ‘*empirical Bayes*’ step was run to derive differential expression results (5) The DEG, defined as those with $\log_2FC > 1$ & Benjamini-Hochberg (BH)-adjusted $p < 0.05$ for up-regulated genes and $\log_2FC < -0.5$ & BH-adjusted $p < 0.05$ for down-regulated genes, were identified for each microarray.

The number of DEG identified by each dataset is shown in **Table 1**. Microarrays with no DEG ($n = 11$) were excluded and those with non-zero DEG ($n = 16$) were visualized with a

stacked bar chart (**Figure 1**). Finally, DEG were classified by tissue types (n = 4; circulatory/ adipose/ digestive/ skeletal muscle) and visualized via a Venn diagram (**Figure 2**). Clinical and other information of the 16 microarrays with non-zero DEG is presented in **S3 Table**. Details of DEG identified by each dataset are presented in **S4 Table**, while DEG classified by tissue type are presented in **S5 Table**.

2.3. Meta-analysis of DEG: Identification of highly perturbed genes

In order to identify highly perturbed genes associated with T2D, we conducted meta-analysis of DEG using ‘*MetaVolcanoR*’ package [39]. We implemented all 3 meta-analytic strategies incorporated in this package, namely, random effects model (REM), vote counting approach (VC) and p-value combining approach (CA).

In brief, REM synthesizes a summary fold change of multiple microarrays based on variance, producing a summary p-value which indicates the probability that the summary fold-change is not different to zero. The ‘*metathr*’ parameter can be specified to filter the desired percentage of the top-most consistently perturbed genes. Gene perturbation is ranked as per the ‘*topconfects*’ approach [40]. The VC algorithm produces DEG according to user-specified p-values and fold change cut-off levels, taking into account both the number of studies in which a DEG appeared and its gene fold change sign consistency. Here also, ‘*metathr*’ parameter can be defined to extract the required percentage of highly perturbed genes. Meta-synthesis of gene perturbation by CA algorithm is at the mean or median level along with p-values derived by Fisher method. A required proportion of top-most DEG can be identified by specifying ‘*metathr*’ parameter with CA as well [39].

As required by the package, the 16 datasets each consisting of the columns: gene name (Symbol), fold change ($\log_2FC$), p-value, and confidence intervals of the fold change (C.I.L & C.I.R) were merged to build a list item. For all 3 meta-analytic models, ‘*metathr*’ was set at

0.01. For VC, p-value and absolute fold change thresholds were set at 0.05 and 0, respectively. Highly perturbed genes identified by each model as well as the compiled list of all highly perturbed genes ($n = 79$) is presented in **Table 2**. Volcano plots illustrating the top-most perturbed genes identified by REM, VC, and CA are given in **Figures 3 – 5**, respectively. Inverse cumulative distribution of consistently differentially expressed genes as per VC was plotted (**Figure 6**) to demonstrate the number of genes with perturbed expression in ≥ 1 studies. We present detailed meta-analytic output from all 3 approaches in **S6 Table** and highly perturbed genes identified by each method in **S7 Table**.

2.4. Network analysis: Identification of hub genes

The list of highly perturbed genes ($n = 79$) was fed into GENEMANIA [41] to determine the gene-gene interactions network. The interactions network formulated by the GENEMANIA gene function prediction program, based on the Multiple Association Network Integration Algorithm (MANIA), incorporates a multitude of functional associations including co-expression, pathways, physical interactions, co-localization, genetic interactions, and protein domain similarity. It has been found more accurate and computationally-efficient than other gene function prediction methods [42, 43]. The gene-gene interactions network was visualized in GENEMANIA (**Figure 7**) while the details of the constructed network were given in **S8 Table**. Next, these interactions were imported to visualize the protein-protein interaction (PPI) network (**Figure 8**) in STRING version 11.0 [44]. We used the ‘*Centiscape*’ application [45] in the ‘*Cytoscape*’ software [46] to analyze the PPI network and determine the hub nodes. After removing nodes with a connection number < 2 , the network was visualized in ‘*Cytoscape*’ (**Figure 9**). Hub genes were defined as those nodes in the network with betweenness, closeness, and degree higher than their mean values. A similar approach has been used in previous studies to demarcate hub genes [27]. Topological features of the 28

hub nodes are summarized in **Table 3** while details of the PPI network derived by ‘Centiscape’ are provided in **S9 Table**.

2.5. Downstream analysis of highly perturbed genes and hub genes

Using ‘Enrichr’ [47-49] platform, we ran a series of downstream analyses for both highly perturbed genes (n = 79) and hub genes (n = 28) as outlined below.

- Ontologies: GO Biological Process 2018 [29]
- Pathways: KEGG 2019 Human [30]
- Diseases/Drugs: COVID-19 Related Gene Sets
- Cell Types: GTEx Tissue Sample Gene Expression Profiles Up & GTEx Tissue Sample Gene Expression Profiles Down
- Miscellaneous: HMDB Metabolites

The Genotype-Tissue Expression (GTEx) portal contains tissue-specific gene expression and regulation data [50], whereas the Human Metabolome Database (HMDB) records human metabolomics data [51]. Findings from downstream analyses of highly perturbed genes are summarized in **Tables 4 – 6** and illustrated in **Figures 10 – 12** while details given in **S10 Table**. Results from downstream analyses of hub genes are presented in **Tables 7 – 9** and visualized in **Figures 13 – 15** with details in **S11 Table**.

3. RESULTS

3.1. A significantly larger proportion of genes associated with T2D is down-regulated.

Of all the DEG identified by different tissues (n = 6284), the proportion of down-regulated genes (n = 4592) were significantly higher than the proportion of upregulated genes (n = 1692) ($p < 0.00000001$). At the level of tissue type, circulatory (255 down-regulated vs 94

up-regulated; $p < 0.000001$), adipose (1426 down-regulated vs 1212 up-regulated; $p = 0.000017$) and digestive (2664 down-regulated vs 135 up-regulated; $p < 0.000001$) tissues revealed a similar pattern while skeletal muscle (247 down-regulated vs 251 up-regulated; $p = 0.44653$) showed no such over-expression of down-regulated genes. Of the 27 microarrays, 11 contained no DEG while the two datasets containing the highest number of DEG were from pancreatic islets (GSE25724; 2164 DEG) and visceral adipose tissue (GSE29231; 1655 DEG) (**Table 1, Figure 1**).

3.2. Tissue-specific DEG are predominant compared to DEG shared between tissues in T2D.

Proportions of tissue-specific DEG were significantly higher than the proportions shared with one or more other tissue types in circulatory (242 specific vs 89 non-specific; $p < 0.000001$), adipose (1989 specific vs 342 non-specific; $p < 0.000001$), and digestive (1875 specific vs 409 non-specific; $p < 0.000001$) groups, while skeletal muscle (244 specific vs 217 non-specific; $p = 0.112959$) had no such over-expression (**Figure 2**).

3.3. Highly perturbed genes associated with T2D comprise both up- and down-regulated genes.

There was no significant difference ($p = 0.410983$) between the proportions of up- (38/79) and down-regulated (41/79) genes that constituted the highly perturbed gene set ($n = 79$) identified by all 3 meta-analytic algorithms (**Table 2, Figures 3 – 6**). The 38 up-regulated genes were: XYLT1, ISLR, CRTAC1, ERAP2, PCOLCE2, VNN2, U2AF2, NPTX2, MMP9, LOC100008589, DYRK3, LOC649456, BCL3, ZNF423, SOD3, CNTFR, ZNF75, RNF19B, TNFAIP6, TAPBP, PHACTR3, PHLDA1, ALDOB, PRIMA1, PVRL2, CNTNAP2, RASL11B, POMZP3, ELFN1, ESPNL, PI3, SCN1B, EGR2, MGRN1, SLC9A3R2, LOC644422, MCL1, and ZBTB16. The 41 down-regulated genes were: LOC650885,

UGT2B7, C19orf33, HLA-DRB4, OR8B12, HLA-DRB5, IFNA7, LOC389286, FUT11, APOL4, LOC731682, KIAA1984, POPDC3, TAP2, CTSC, DYRK2, MCOLN3, TMEM37, API5, ARG2, C14orf132, COG2, DHRS2, ENPP2, ENTPD3, HADH, KIAA1279, LARP4, MARK1, MTRR, NAALAD2, PAAF1, PPM1K, PPP1R1A, RPL14, SLC2A2, SNAP25, STMN2, NMNAT2, ASCL2, and IAPP.

3.4. Hub genes associated with T2D also comprise both up- and down-regulated genes.

The 28 hub genes consisted of 13 up-regulated, 9 down-regulated and 6 predicted genes. There was no significant difference ($p = 0.261216$) between the proportions of up- (13/22) and down-regulated (9/22) genes constituting the hub gene set. The 13 up-regulated hub genes were: PHLDA1, ZBTB16, SOD3, ISLR, SCN1B, MMP9, MCL1, CNTNAP2, ZNF423, CNTFR, TNFAIP6, XYLT1, and RASL11B. The 9 down-regulated genes were: CTSC, HADH, RPL14, SNAP25, SLC2A2, ENPP2, STMN2, PPP1R1A, and UGT2B7. The 6 hub genes predicted by GENEMANIA were: TAP1, DPEP1, PPP1R15A, ZMIZ1, ARG1, and CD226 (**Table 3**).

3.5. Enriched biological processes associated with highly perturbed genes and hub genes of T2D.

Ontology analysis of highly perturbed genes identified 89 enriched biological processes (**S10 Table**). Top 10 enriched biological processes were: Intrinsic apoptotic signaling pathway in response to DNA damage (GO:0008630); Intrinsic apoptotic signaling pathway in response to DNA damage by p53 class mediator (GO:0042771); Antigen processing and presentation of peptide antigen via MHC class I (GO:0002474); Cellular response to oxidative stress (GO:0034599); Plasma membrane bounded cell projection organization (GO:0120036); Intrinsic apoptotic signaling pathway by p53 class mediator (GO:0072332); Myeloid cell

differentiation (GO:0030099); Nicotinamide nucleotide metabolic process (GO:0046496); Regulation of intrinsic apoptotic signaling pathway (GO:2001242); and Myeloid leukocyte differentiation (GO:0002573). Three of these: Antigen processing and presentation of peptide antigen via MHC class I (GO:0002474); Myeloid cell differentiation (GO:0030099); and Regulation of intrinsic apoptotic signaling pathway (GO:2001242) were associated with up-regulated genes in T2D (**Table 4**).

Ontological analysis of hub genes identified 179 enriched biological processes (**S11 Table**). Top 10 enriched biological processes were: Neutrophil degranulation (GO:0043312); Neutrophil activation involved in immune response (GO:0002283); Neutrophil mediated immunity (GO:0002446); Regulation of intrinsic apoptotic signaling pathway (GO:2001242); Negative regulation of intrinsic apoptotic signaling pathway (GO:2001243); Positive regulation of cell projection organization (GO:0031346); Cellular response to reactive oxygen species (GO:0034614); Negative regulation of apoptotic signaling pathway (GO:2001234); Negative regulation of cysteine-type endopeptidase activity involved in apoptotic process (GO:0043154); and Regulation of peptide hormone secretion (GO:0090276). Of these, three [Regulation of intrinsic apoptotic signaling pathway (GO:2001242); Negative regulation of intrinsic apoptotic signaling pathway (GO:2001243); Negative regulation of apoptotic signalling pathway (GO:2001234)] were associated with up-regulated genes in T2D whereas one [Regulation of peptide hormone secretion (GO:0090276)] was associated with down-regulated genes in T2D (**Table 7**).

3.6. Enriched KEGG pathways associated with highly perturbed genes and hub genes of T2D.

As per KEGG analysis of highly perturbed genes, 21 pathways were enriched (**S10 Table**). The top 10 pathways were: Epstein-Barr virus infection; Antigen processing and presentation;

Autoimmune thyroid disease; Maturity onset diabetes of the young; Asthma; Allograft rejection; Graft-versus-host disease; Type 1 diabetes mellitus; Intestinal immune network for IgA production; and Cell adhesion molecules (CAMs). Seven of these [Autoimmune thyroid disease; Maturity onset diabetes of the young; Asthma; Allograft rejection; Graft-versus-host disease; Type 1 diabetes mellitus; Intestinal immune network for IgA production] were associated with down-regulated genes in T2D (**Table 4**).

According to KEGG analysis of hub genes, 12 pathways were enriched (**S11 Table**). The top 10 pathways were: Insulin secretion; Apoptosis; Adrenergic signaling in cardiomyocytes; Cell adhesion molecules (CAMs); JAK-STAT signaling pathway; Transcriptional mis-regulation in cancer; Arginine biosynthesis; Maturity onset diabetes of the young; Ascorbate and aldarate metabolism; and Fatty acid elongation. Four of these [Insulin secretion; Maturity onset diabetes of the young; Ascorbate and aldarate metabolism; Fatty acid elongation] were associated with down-regulated genes in T2D while two pathways [JAK-STAT signaling pathway and Transcriptional mis-regulation in cancer] were associated with up-regulated genes in T2D (**Table 7**).

3.7. COVID-19 related gene sets associated with highly perturbed genes and hub genes of T2D.

Downstream analyses revealed 20 COVID-19 related gene sets associated with the highly perturbed genes of T2D (**S10 Table**) and the top 10 of these are visualized in **Figure 11 (a)**. There were 23 COVID-19 related gene sets associated with the hub genes of T2D (**S11 Table**), the top 10 of which are visualized in **Figure 14 (a)**.

3.8. HMDB metabolites associated with highly perturbed genes and hub genes of T2D.

Four HMDB metabolites [Zinc (HMDB01303), Manganese (HMDB01333), Magnesium (HMDB00547), C₁₀H₁₃N₂O₇P (HMDB01570)] were associated with the highly perturbed genes of T2D (**S10 Table**). Of these, Zinc (HMDB01303) was associated with up-regulated genes in T2D while C₁₀H₁₃N₂O₇P (HMDB01570) was associated with down-regulated genes in T2D (**Table 5**).

There were 45 HMDB Metabolites associated with the hub genes of T2D (**S11 Table**). The top 10 metabolites were: Zinc (HMDB01303); Ethyl glucuronide (HMDB10325); 3-Acetoacetyl-CoA (HMDB01484); (S)-Methylmalonate semialdehyde (HMDB02217); C₁₈H₃₁NO₁₄S (HMDB00632); Ornithine (HMDB00214); C₂₄H₃₂O₈ (HMDB06224); C₂₄H₃₂O₉ (HMDB10354); 11beta,18-Epoxy-18,21-dihydroxy-20-oxo-5beta-pregnan-3alpha-yl beta-D-glucopyranosiduronic acid (HMDB10357) and C₂₅H₄₀O₈ (HMDB10359). Seven of these [Ethyl glucuronide (HMDB10325); 3-Acetoacetyl-CoA (HMDB01484); (S)-Methylmalonate semialdehyde (HMDB02217); C₂₄H₃₂O₈ (HMDB06224); C₂₄H₃₂O₉ (HMDB10354); C₂₅H₄₀O₈ (HMDB10359)] and 11beta,18-Epoxy-18,21-dihydroxy-20-oxo-5beta-pregnan-3alpha-yl beta-D-glucopyranosiduronic acid (HMDB10357) were associated with down-regulated genes in T2D. One metabolite [C₁₈H₃₁NO₁₄S (HMDB00632)] was associated with upregulated genes of T2D (**Table 8**).

3.9. Gene expression in different cell types associated with highly perturbed genes and hub genes of T2D.

There were 168 down-regulated GTEx profiles (**S10 Table**) associated with highly perturbed genes of T2D, the top 10 of which consisted of one brain, two thyroid, and seven blood tissue samples (**Table 6 & Figure 12**). There were 77 up-regulated GTEx profiles (**S10 Table**) associated with highly perturbed genes of T2D, the top 10 of which consisted of six adipose- and one each of lung, breast, heart, and blood vessel tissue samples (**Table 6 & Figure 12**).

There were 223 down-regulated GTEx profiles (**S11 Table**) associated with hub genes of T2D, the top 10 of which consisted of five nervous system (three brain, one nerve, one pituitary), three blood, one breast and one bladder tissue samples (**Table 9 & Figure 15**). There were 178 up-regulated GTEx profiles (**S11 Table**) associated with hub genes of T2D, the top 10 of which consisted of six adipose-, two blood and two skin tissue samples (**Table 9 & Figure 15**).

4. DISCUSSION

In this study, we identified highly perturbed genes and hub genes associated with T2D in different tissues of adult humans, via an extensive bioinformatics analytic workflow. By doing this, we revealed valuable insights with respect to T2D pathogenesis, including associations with other diabetes phenotypes and COVID-19, patterns of tissue-specific and tissue non-specific differential gene expression as well as pathophysiological manifestations such as those related to insulin action, immunity, and apoptosis. Salient findings of the study which contribute towards the understanding of the genetic basis of T2D are further discussed below. The comprehensive evidence synthesis approach with open-source gene expression data exemplified in this study can be replicated to gain high-level evidence synthesis for other clinical conditions.

4.1. Patterns of differential gene expression in T2D

Our findings indicate that T2D seems rather a disorder of gene down-regulation than up-regulation, when the whole genome is considered. This is consistent with previous studies where a preponderance of gene down-regulation was associated with T2D (52, 53). Also, hyperglycemia-induced global downregulation of gene expression in adipose and skeletal muscle tissues have been documented previously (54). A similar pattern has been observed in T1D (55) as well as other endocrine disorders such as polycystic ovary syndrome (56). In

contrast, highly perturbed genes and hub genes associated with T2D, which might together constitute the candidate gene set critical for pathogenicity of T2D, were found to contain both up- and down-regulated genes. This presentation suggests a more complex dysregulation at the crux of the GRN of T2D, involving actions and interactions between both repressed and augmented genes.

4.2. Tissue-specific and tissue non-specific DEG associated with T2D

Results of the present study indicate the predominance of tissue-specific DEG in T2D which may have important implications for guiding biomarker discovery process. It supports the use of target tissue gene expression analysis as a viable avenue for identifying tissue-specific T2D biomarkers. A previous analysis integrating multiple tissue transcriptomics and PPI data to explore molecular biomarkers of T2D confirmed the presence of common signatures (57). We also observed common DEG across different tissue types which can act as confluent molecular signatures of T2D. Identification of tissue-specific and non-specific molecular gene signatures of T2D facilitates downstream exploration of key pathways amenable to therapeutic targeting and drug repurposing efforts.

4.3. Shared gene enrichment across diabetes phenotypes

As revealed by KEGG pathway analyses, both MODY and T1D were enriched pathways associated with highly perturbed genes of T2D. In addition, MODY was an enriched pathway associated with hub genes of T2D as well. Specifically, SLC2A2 (GLUT2) and IAPP genes were commonly enriched in MODY, while HLA-DRB4 and HLA-DRB5 genes were underlying the enrichment with T1D. A gene expression meta-analysis also revealed the existence of possible pleiotropic mechanisms manifest via common gene signatures (PGRMC1 and HADH) across different diabetes phenotypes [35].

Down-regulation of SLC2A2 is associated with not only T2D [58] but also neonatal diabetes [59] and early childhood diabetes [60] suggesting a likely role in insulin secretion. Amylin (IAPP), a gluco-modulatory hormone co-expressed with insulin by pancreatic β cells, is down-regulated in both T1D and advanced T2D [61] while amylin agonists are considered as novel therapeutic agents for treating diabetes [62]. It has also been found that human amylin plays a protective role against autoimmune diabetes inducing CD4+Foxp3+ regulatory T cells [63]. Downregulation of HLA-DRB4 in peripheral blood mononuclear cells (PBMC) is associated with T2D as well as dyslipidemia and periodontitis [64], while a meta-analysis revealed that the lack of HLA-DRB5 increased T2D risk [65]. Intriguingly, both HLA-DRB4 and HLA-DRB5 are associated with β cell autoantibodies and T1D [66]. In fact, previous studies reported that both T1D and T2D share HLA class II locus components [65]. Interestingly, two of the hub genes of T2D found in our study (MMP9, ARG1) have been found as hub genes of T1D in a previous analysis [67]. Collectively, these findings support some degree of shared genetic architecture between T2D and other diabetes pathologies.

4.4. T2D as a disorder of insulin secretion and action

Downstream analyses provided insights into the characterization of T2D as a disorder of insulin secretion and action. We found that insulin secretion was the most significant KEGG pathway associated with hub genes, whereby two down-regulated hub genes (SLC2A2, SNAP25) in T2D were underlying this enrichment. Zinc, the most significant HMDB metabolite associated with both highly perturbed genes and hub genes of T2D, is an essential element with key regulatory roles in insulin synthesis, storage, and secretion [68]. Other metabolomic biomarkers associated with highly perturbed genes included magnesium which is necessary for insulin signaling [69] as well as manganese which is involved in insulin synthesis and secretion [70]. Together, these findings underscore the effects on insulin production and action as pivotal to T2D pathogenesis.

4.5. Pathophysiological manifestations of T2D

4.5.1. Apoptosis

Downstream analyses revealed that multiple GO and KEGG pathways associated with apoptosis, including intrinsic apoptotic signaling pathway, were enriched in T2D. It is known that hyperglycemia-induced β cell apoptosis, a hallmark in T2D progression, occurs via intrinsic pathways causing reduced islet mass and metabolic abnormalities [71]. Hyperglycemia-induced apoptosis has been reported to occur in other sites such as renal cells [72] and coronary arteries [73], indicating a possible role in disease progression and the onset of complications.

4.5.2. Immunity

Downstream analyses also revealed that multiple immunity-related GO and KEGG pathways, encompassing both innate and humoral immune responses, were enriched in T2D. These included multiple ontologies involving neutrophils, antigen processing and presentation as well as severe immune reactions such as graft-versus-host disease and allograft rejection. Impaired immunity in T2D and consequent susceptibility to infections and complications is frequently observed [74]. A deeper understanding of the genomics underlying impaired immunity in T2D might provide opportunities to personalize the management of co-morbidities and pharmacotherapy.

4.6. COVID-19 and T2D

Epidemiological studies strongly suggest poorer prognosis of COVID-19 among people with T2D [75], although underlying mechanisms are not well-understood [76]. Downstream analysis of highly perturbed genes and hub genes of T2D in the present study revealed a large number of enriched COVID-19 related gene sets, providing support for this putative link at a more granular level.

4.7. Over- and under- expression of genes in different tissues associated with T2D

Downstream analysis of GTEx profiles identified tissues that are likely to demonstrate under- and over-expression of DEG associated with T2D. Findings indicate that adipose tissue tends to over-express marker genes of T2D, while these might be under-expressed in other tissues such as those of the nervous system. These findings have implications for biomarker discovery and can guide further research on tissues which should be explored for DEG identification.

5. CONCLUSIONS

Taken together, these findings contribute towards the understanding of the genetic basis of T2D, which is fundamental for precision medicine approaches for individualized T2D care. Further research is warranted to explore and substantiate the molecular mechanisms underlying these findings which would be critical for establishing precision T2D medicine initiatives. The proposed bioinformatics pipeline may have broader use as a judicious strategy to identify gene perturbations and pathophysiological mechanisms of other clinical conditions beyond T2D which ought to be validated in future research. Finally, this study describes an exemplary approach to applying comprehensive evidence synthesis using existing open source gene expression data. Other researchers are encouraged to apply this methodology to obtain high-level evidence from existing multiple datasets thereby getting the most value from existing bioinformatics sources.

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Availability of data and material: Data used in this study are freely available at the National Center for Biotechnology Information Gene Expression Omnibus (NCBI GEO) portal: <https://www.ncbi.nlm.nih.gov/geo/>

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TABLES

Table 1: Number of differentially expressed genes in type 2 diabetes identified from different tissues of adult humans (n = 27)

Dataset	Number of DEGs^a (n = 6284)	Upregulated^b (n = 1692)	Downregulated^c (n = 4592)	Tissue
GSE156993	0	0	0	PBMC
GSE15932	309	79	230	Peripheral blood
GSE21321	37	15	22	Peripheral blood
GSE13015	3	0	3	Whole blood
GSE13760	0	0	0	Arterial tissue
GSE78721	1	0	1	Adipocytes
GSE71416	13	0	13	Omental adipose tissue
GSE54350	2	2	0	Visceral adipose tissue
GSE29231	1655	1051	604	Visceral adipose tissue
GSE16415	0	0	0	Visceral adipose tissue
GSE29226	967	159	808	Subcutaneous adipose tissue
GSE27949	0	0	0	Subcutaneous adipose tissue
GSE76895	101	31	70	Pancreatic islets
GSE76894	478	2	476	Pancreatic islets
GSE38642	0	0	0	Pancreatic islets
GSE25724	2164	76	2088	Pancreatic islets
GSE20966	56	26	30	Beta cells from pancreatic tissue
GSE64998	0	0	0	Liver
GSE23343	0	0	0	Liver
GSE15653	0	0	0	Liver
GSE73034	0	0	0	Skeletal muscle
GSE55650	30	9	21	Skeletal muscle
GSE29221	185	26	159	Skeletal muscle
GSE25462	8	6	2	Skeletal muscle

GSE19420	0	0	0	Skeletal muscle
GSE22309	275	210	65	Skeletal muscle
GSE21340	0	0	0	Skeletal muscle

a = differentially expressed genes were defined as BH-adjusted p-value < 0.05 & $\log_2FC > 1$ | $\log_2FC < -0.5$; b = Upregulated genes were defined as BH-adjusted p-value < 0.05 & $\log_2FC > 1$; c = Downregulated genes were defined as BH-adjusted p-value < 0.05 & $\log_2FC < -0.5$.

DEGs = differentially expressed genes; PBMC = peripheral blood mononuclear cells.

Table 2: Highly perturbed genes (n = 79) associated with type 2 diabetes identified by meta-analysis of microarray (n = 16).

Approach	Highly perturbed genes	Up-regulated genes	Down-regulated genes
Random-effects model	49 genes: XYLT1, ISLR, CRTAC1, ERAP2, PCOLCE2, VNN2, U2AF2, NPTX2, MMP9, LOC100008589, DYRK3, LOC649456, BCL3, ZNF423, SOD3, CNTFR, ZNF75, RNF19B, TNFAIP6, TAPBP, PHACTR3, PHLDA1, ALDOB, PRIMA1, PVRL2, CNTNAP2, RASL11B, POMZP3, ELFN1, ESPNL, PI3, SCN1B, EGR2, MGRN1, SLC9A3R2, LOC650885, UGT2B7, C19orf33, HLA-DRB4, OR8B12, HLA-DRB5, IFNA7, LOC389286, FUT11, APOL4, LOC731682, KIAA1984, POPDC3, TAP2	35 genes: XYLT1, ISLR, CRTAC1, ERAP2, PCOLCE2, VNN2, U2AF2, NPTX2, MMP9, LOC100008589, DYRK3, LOC649456, BCL3, ZNF423, SOD3, CNTFR, ZNF75, RNF19B, TNFAIP6, TAPBP, PHACTR3, PHLDA1, ALDOB, PRIMA1, PVRL2, CNTNAP2, RASL11B, POMZP3, ELFN1, ESPNL, PI3, SCN1B, EGR2, MGRN1, SLC9A3R2	14 genes: LOC650885, UGT2B7, C19orf33, HLA-DRB4, OR8B12, HLA-DRB5, IFNA7, LOC389286, FUT11, APOL4, LOC731682, KIAA1984, POPDC3, TAP2
Vote-counting approach	27 genes: LOC644422, MCL1, CTSC, DYRK2, MCOLN3, TMEM37, API5, ARG2, C14orf132, COG2, DHRS2, ENPP2, ENTPD3, HADH, KIAA1279, LARP4, MARK1, MTRR, NAALAD2, PAAF1, PPM1K, PPP1R1A, RPL14, SLC2A2, SNAP25, STMN2, NMNAT2	2 genes: LOC644422, MCL1	25 genes: CTSC, DYRK2, MCOLN3, TMEM37, API5, ARG2, C14orf132, COG2, DHRS2, ENPP2, ENTPD3, HADH, KIAA1279, LARP4, MARK1, MTRR, NAALAD2, PAAF1, PPM1K, PPP1R1A, RPL14, SLC2A2, SNAP25, STMN2, NMNAT2
Combining approach	8 genes: PCOLCE2, ERAP2, EGR2, ZBTB16, SLC2A2, ARG2, ASCL2, IAPP	4 genes: PCOLCE2, ERAP2, EGR2, ZBTB16	4 genes: SLC2A2, ARG2, ASCL2, IAPP
Compiled set	79 genes: XYLT1, ISLR, CRTAC1, ERAP2, PCOLCE2, VNN2, U2AF2, NPTX2, MMP9, LOC100008589, DYRK3, LOC649456, BCL3, ZNF423, SOD3, CNTFR, ZNF75, RNF19B, TNFAIP6, TAPBP, PHACTR3, PHLDA1,	38 genes: XYLT1, ISLR, CRTAC1, ERAP2, PCOLCE2, VNN2, U2AF2, NPTX2, MMP9, LOC100008589, DYRK3, LOC649456, BCL3, ZNF423, SOD3, CNTFR, ZNF75, RNF19B,	41 genes: LOC650885, UGT2B7, C19orf33, HLA-DRB4, OR8B12, HLA-DRB5, IFNA7, LOC389286, FUT11, APOL4, LOC731682, KIAA1984, POPDC3, TAP2, CTSC, DYRK2, MCOLN3, TMEM37, API5, ARG2,

ALDOB, PRIMA1, PVRL2, CNTNAP2, TNFAIP6, TAPBP, PHACTR3, C14orf132, COG2, DHRS2, ENPP2, RASL11B, POMZP3, ELFN1, ESPNL, PHLDA1, ALDOB, PRIMA1, ENTPD3, HADH, KIAA1279, LARP4, PI3, SCN1B, EGR2, MGRN1, PVRL2, CNTNAP2, RASL11B, MARK1, MTRR, NAALAD2, PAAF1, SLC9A3R2, LOC644422, MCL1, POMZP3, ELFN1, ESPNL, PI3, PPM1K, PPP1R1A, RPL14, SLC2A2, ZBTB16, LOC650885, UGT2B7, SCN1B, EGR2, MGRN1, SNAP25, STMN2, NMNAT2, ASCL2, C19orf33, HLA-DRB4, OR8B12, HLA-DRB5, IFNA7, LOC389286, FUT11, SLC9A3R2, LOC644422, MCL1, IAPP, ZBTB16, APOL4, LOC731682, KIAA1984, POPDC3, TAP2, CTSC, DYRK2, MCOLN3, TMEM37, API5, ARG2, C14orf132, COG2, DHRS2, ENPP2, ENTPD3, HADH, KIAA1279, LARP4, MARK1, MTRR, NAALAD2, PAAF1, PPM1K, PPP1R1A, RPL14, SLC2A2, SNAP25, STMN2, NMNAT2, ASCL2, IAPP

Table 3: Topological characteristics of the hub genes (n = 28) identified via network analyses

Gene	Betweenness	Closeness	Degree	Regulation
	mean = 115.5333333	mean = 0.004988249	mean = 12.68888889	
PHLDA1	213.0074	0.005618	22	Up-regulated
CTSC	149.7385	0.005495	18	Down-regulated
HADH	208.3918	0.00578	18	Down-regulated
RPL14	139.3092	0.005464	17	Down-regulated
ZBTB16	127.1864	0.005405	14	Up-regulated
SOD3	198.8821	0.005714	21	Up-regulated
SNAP25	380.1873	0.005814	30	Down-regulated
SLC2A2	438.8331	0.005747	26	Down-regulated
ISLR	465.6411	0.005952	27	Up-regulated
SCN1B	175.7841	0.005376	14	Up-regulated
MMP9	196.9649	0.005682	22	Up-regulated
ENPP2	230.6537	0.005618	17	Down-regulated
TAP1*	189.4171	0.005376	40	-
MCL1	320.3027	0.005917	27	Up-regulated
DPEP1*	133.0483	0.005208	15	-
CNTNAP2	761.7546	0.006494	39	Up-regulated
STMN2	200.277	0.005618	18	Down-regulated
PPP1R1A	219.8601	0.005587	18	Down-regulated
ZNF423	406.5439	0.006061	21	Up-regulated
CNTFR	200.657	0.005319	16	Up-regulated
TNFAIP6	162.3969	0.005208	14	Up-regulated
PPP1R15A*	119.5084	0.005155	13	-
XYLT1	194.4998	0.00565	18	Up-regulated
ZMIZ1*	392.1699	0.005682	26	-

RASL11B	168.9444	0.005376	13	Up-regulated
ARG1*	124.961	0.005263	18	-
UGT2B7	247.0102	0.005682	19	Down-regulated
CD226*	222.4953	0.005495	17	-

*Genes included in the network as predicted by GENEMANIA

Table 4: Downstream analyses of highly perturbed genes (n = 79) associated with type 2 diabetes in different tissues of human adults: GO biological processes and KEGG pathways

Term	Overlap	P-value	Adjusted P-value	Odds Ratio	Combined Score	Genes	Regulation in T2D
Ontologies: GO biological processes							
Intrinsic apoptotic signalling pathway in response to DNA damage (GO:0008630)	3/48	9.03E-04	0.230983	17.43509	122.2228	DYRK2; BCL3; MCL1	-
Intrinsic apoptotic signalling pathway in response to DNA damage by p53 class mediator (GO:0042771)	2/20	0.002795	0.230983	28.72006	168.8714	DYRK2; BCL3	-
Antigen processing and presentation of peptide antigen via MHC class I (GO:0002474)	2/28	0.005448	0.230983	19.87512	103.5986	ERAP2; TAPBP	Up
Cellular response to oxidative stress (GO:0034599)	3/115	0.010668	0.230983	6.981555	31.69995	MMP9; DHRS2; SOD3	-
Plasma membrane bounded cell projection organization (GO:0120036)	3/118	0.011436	0.230983	6.798398	30.39569	CNTNAP2; STMN2; NPTX2	-
Intrinsic apoptotic signalling pathway by p53 class mediator (GO:0072332)	2/43	0.012527	0.230983	12.59424	55.1606	DYRK2; BCL3	-
Myeloid cell differentiation (GO:0030099)	2/44	0.013091	0.230983	12.29375	53.30386	DYRK3; ZBTB16	Up
Nicotinamide nucleotide metabolic process (GO:0046496)	2/45	0.013665	0.230983	12.00725	51.54622	NMNAT2; ALDOB	-
Regulation of intrinsic apoptotic signalling pathway (GO:2001242)	2/47	0.014845	0.230983	11.47244	48.2997	MMP9; MCL1	Up
Myeloid leukocyte differentiation (GO:0002573)	2/50	0.016695	0.230983	10.75379	44.01114	MMP9; DHRS2	-
Pathways: KEGG							
Epstein-Barr virus infection	6/201	0.000144	0.012503	8.31444	73.51986	IFNA7; HLA-DRB5; HLA-DRB4; ENTPD3; TAP2; TAPBP	-

Antigen processing and presentation	4/77	0.000245	0.012503	14.50082	120.5547	HLA-DRB5; HLA-DRB4; TAP2; TAPBP	-
Autoimmune thyroid disease	3/53	0.001205	0.040983	15.68763	105.436	IFNA7; HLA-DRB5; HLA-DRB4	Down
Maturity onset diabetes of the young (MODY)	2/26	0.004708	0.12006	21.53355	115.3862	SLC2A2; IAPP	Down
Asthma	2/31	0.006651	0.135681	17.81639	89.31323	HLA-DRB5; HLA-DRB4	Down
Allograft rejection	2/38	0.009878	0.159725	14.34704	66.24726	HLA-DRB5; HLA-DRB4	Down
Graft-versus-host disease	2/41	0.011434	0.159726	13.24142	59.20461	HLA-DRB5; HLA-DRB4	Down
Type I diabetes mellitus (T1DM)	2/43	0.012527	0.159726	12.59423	55.1606	HLA-DRB5; HLA-DRB4	Down
Intestinal immune network for IgA production	2/48	0.015452	0.175118	11.22247	46.79817	HLA-DRB5; HLA-DRB4	Down
Cell adhesion molecules (CAMs)	3/145	0.019771	0.181561	5.49824	21.5726	CNTNAP2; HLA-DRB5; HLA-DRB4	-

GO = gene ontology; KEGG = Kyoto Encyclopedia of Genes and Genomes; T2D = type 2 diabetes

Table 5: Downstream analyses of highly perturbed genes (n = 79) associated with type 2 diabetes in different tissues of human adults: COVID-19 related gene sets and HMDB metabolites

Term	Overlap	P-value	Adjusted P-value	Odds Ratio	Combined Score	Genes	Regulation in T2D
Diseases: COVID-19 Related Gene Sets							
Up-regulated by SARS-CoV-2 in pancreatic organoids from GSE151803	9/500	0.000155	0.01841	5.08787	44.64083	TMEM37; PPP1R1A; VNN2; BCL3; TAP2; ELFN1; MMP9; DHRS2; SCN1B	-
SARS Perturbation Down Genes Mouse Lung from GSE19137:GPL1261:2	5/246	0.002908	0.08566	5.51755	32.22428	PCOLCE2; RPL14; HADH; CTSC; MCL1	-
SARS Perturbation Up Genes Mouse Lung from GSE19137:GPL1261:3	6/366	0.003262	0.08566	4.46598	25.56907	SLC9A3R2; HLA-DRB5; U2AF2; ZBTB16; RPL14; MCL1	-
Healthy lung biopsy vs. COVID-19 infected lung series 15 from GSE147507 up genes	7/500	0.003599	0.08566	3.8313	21.55896	POPDC3; HLA-DRB5; TNFAIP6; VNN2; BCL3; RNF19B; CTSC	-
Up-regulated by SARS-CoV-2 in lung tissue from GSE147507	7/500	0.003599	0.08566	3.8313	21.55896	POPDC3; HLA-DRB5; TNFAIP6; VNN2; BCL3; RNF19B; CTSC	-
SARS-CoV perturbation Up Genes bronchial epithelial 2B4 from GSE17400:GPL570:6	6/402	0.005143	0.100484	4.05251	21.35735	ERAP2; VNN2; TAP2; RNF19B; PPM1K; DHRS2	-
SARS Perturbation Up Genes Mouse Lung from GSE19137:GPL1261:5	5/291	0.005911	0.100484	4.63877	23.80141	HLA-DRB5; U2AF2; ZBTB16; RPL14; MCL1	-
Up-regulated by SARS-CoV-1 in Calu-3 from GSE148729	6/498	0.013921	0.16867	3.24574	13.87347	APOL4; EGR2; TAP2; RNF19B; PPM1K; DHRS2	-
Up-regulated by SARS-CoV-2 in Calu-3 24hr from GSE148729	6/499	0.014047	0.16867	3.23899	13.81541	APOL4; EGR2; ERAP2; TAP2; RNF19B; PPM1K	-

Up-regulated by SARS-CoV-2 in NHBE from GSE147507	6/500	0.014174	0.16867	3.23227	13.75766	ENTPD3; BCL3; TAP2; PI3; MMP9; CTSC	-
Miscellaneous: HMDB Metabolites							
Zinc (HMDB01303)	4/82	0.000312	0.025909	13.56786	109.52001	ZBTB16; ALDOB; MMP9; SOD3	Up
Manganese (HMDB01333)	4/193	0.007193	0.127982	5.56811	27.4768	ARG2; DYRK2; PPM1K; SOD3	-
Magnesium (HMDB00547)	6/463	0.009991	0.127982	3.50061	16.12387	DYRK3; DYRK2; ENTPD3; PPM1K; NMNAT2; MARK1	-
C10H13N2O7P (HMDB01570)	1/11	0.042612	0.127982	25.52692	80.55315	ENTPD3	Down

Table 6: Downstream analyses of highly perturbed genes (n = 79) associated with type 2 diabetes in different tissues of human adults: GTEx tissue sample gene expression profiles.

Term	Overlap	P-value	Adjusted P-value	Odds Ratio	Combined Score	Genes	Regulation in T2D
Cell Types: GTEx Tissue Sample Gene Expression Profiles Down							
GTEx-XUJ4-0726-SM-4BOOP thyroid female 60-69 years	4/79	0.00027	0.3129	14.1127	115.93606	HLA-DRB5; STMN2; RNF19B; DHRS2	-
GTEx-V955-0004-SM-3NMDH blood male 60-69 years	23/2789	0.00034	0.3129	2.54729	20.28253	CNTFR; POPDC3; RASL11B; PCOLCE2; ZBTB16; C14ORF132; NMNAT2; ASCL2; SOD3; POMZP3; SLC9A3R2; APOL4; ISLR; PPP1R1A; CRTAC1; PHACTR3; PI3; NPTX2; ZNF423; PVRL2; SCN1B; PRIMA1; MARK1	-
GTEx-XXEK-0004-SM-4BRWO blood male 50-59 years	24/3085	0.00059	0.3129	2.40349	17.85933	CNTFR; CNTNAP2; SNAP25; POPDC3; ARG2; RASL11B; ENTPD3; PCOLCE2; ZBTB16; XYLT1; C14ORF132; NMNAT2; ELFN1; ASCL2; SOD3; SLC9A3R2; ISLR; PPP1R1A; CRTAC1; NPTX2; ZNF423; PVRL2; SCN1B; MARK1	-
GTEx-S7SE-0005-SM-2XCEA blood male 50-59 years	23/2919	0.00067	0.3129	2.41451	17.63845	POPDC3; ARG2; RASL11B; ENTPD3; PCOLCE2; NMNAT2; LARP4; ELFN1; SOD3; SLC9A3R2; TMEM37; ISLR; ESPNL; KIAA1279; ENPP2; PHACTR3; HADH; NPTX2; ZNF423; PVRL2; SCN1B; PRIMA1; MARK1	-
GTEx-XUYS-0005-SM-47JZ2 blood male 50-59 years	24/3115	0.00068	0.3129	2.37593	17.31639	CNTFR; SNAP25; POPDC3; DYRK3; RASL11B; ENTPD3; PCOLCE2; ZBTB16; NMNAT2; LARP4; SOD3; SLC9A3R2; TMEM37; ISLR; KIAA1279; PPP1R1A; CRTAC1; ENPP2; HADH;	-

GTEX-XUJ4-0004-SM-4BOQE blood female 60-69 years	25/3348	0.00081	0.3129	2.31245	16.45932	NPTX2; ZNF423; PHLDA1; PVRL2; MARK1 SNAP25; PCOLCE2; STMN2; ELFN1; ASCL2; SLC9A3R2; APOL4; ISLR; CRTAC1; PHACTR3; PI3; ZNF423; PHLDA1; SCN1B; PRIMA1; MARK1; ARG2; ENTPD3; ZBTB16; C14ORF132; NMNAT2; SOD3; PPP1R1A; ALDOB; PVRL2	-
GTEX-WL46-2826-SM-3LK81 brain male 50-59 years	17/1966	0.00149	0.33149	2.52838	16.44486	DYRK3; EGR2; MCOLN3; DYRK2; TNFAIP6; PCOLCE2; ERAP2; MMP9; ASCL2; SOD3; APOL4; ISLR; VNN2; BCL3; PVRL2; CTSC; MCL1	-
GTEX-TML8-0001-SM-3NMAF blood female 40-49 years	22/2914	0.00161	0.33149	2.27268	14.60663	CNTFR; RASL11B; ENTPD3; PCOLCE2; ZBTB16; STMN2; C14ORF132; NMNAT2; ASCL2; SOD3; SLC9A3R2; APOL4; ISLR; PPP1R1A; CRTAC1; ALDOB; ZNF423; PHLDA1; PVRL2; SCN1B; PRIMA1; MARK1	-
GTEX-OIZF-0006-SM-2I5GQ blood male 60-69 years	32/5000	0.00172	0.33149	2.04927	13.03763	CNTFR; POPDC3; DYRK3; MTRR; RASL11B; ELFN1; POMZP3; SLC9A3R2; APOL4; ISLR; CRTAC1; ENPP2; NAALAD2; HADH; NPTX2; ZNF423; PHLDA1; PAAF1; PRIMA1; MARK1; EGR2; MCOLN3; ENTPD3; ZBTB16; C14ORF132; NMNAT2; LARP4; SOD3; TMEM37; KIAA1279; PPP1R1A; PVRL2	-
GTEX-WFJO-0226-SM-3GIKW thyroid male 30-39 years	5/222	0.00186	0.33149	6.13526	38.55908	SNAP25; TNFAIP6; STMN2; ALDOB; PI3	-
Cell Types: GTEx Tissue Sample Gene Expression Profiles Up							
GTEX-WHPG-0626-SM-3NMBD adipose tissue male 50-59 years	12/778	0.00005	0.147791	4.47878	44.22047	CNTFR; TMEM37; APOL4; DYRK3; EGR2; HLA-DRB5; PCOLCE2; BCL3; ENPP2; HADH; ZNF423; MCL1	-
GTEX-XGQ4-1026-SM-4AT4L adipose tissue male 50-59 years	9/598	0.00057	0.448488	4.21994	31.48829	SLC9A3R2; CNTFR; APOL4; EGR2; PPP1R1A; BCL3; HADH; ELFN1; MCL1	-
GTEX-X88G-0226-SM-4GIE4 adipose tissue male 30-39 years	11/872	0.00061	0.448488	3.58099	26.50301	TMEM37; APOL4; ISLR; EGR2; TNFAIP6; PCOLCE2; ERAP2; BCL3; TAP2; CTSC; MCL1	-
GTEX-WHPG-1426-SM-3NMBB lung male 50-59 years	13/1178	0.00067	0.44848	3.17113	23.17132	HLA-DRB5; MTRR; TAP2; RNF19B; MMP9; TAPBP; SLC9A3R2; APOL4; VNN2; CRTAC1; BCL3; CTSC; MCL1	-
GTEX-WH7G-1126-SM-3NMBK adipose tissue male 40-49 years	10/758	0.00078	0.448488	3.71483	26.5752	CNTFR; APOL4; DYRK3; TNFAIP6; PCOLCE2; PPP1R1A; BCL3; HADH; ELFN1; MCL1	-
GTEX-P4PP-3026-SM-3P61O blood vessel female 30-39	7/434	0.00163	0.71722	4.43852	28.4846	SLC9A3R2; CNTFR; APOL4; EGR2; RASL11B; PCOLCE2; ZNF423	-

years GTEX-PWCY- 1926-SM-3NB25 adipose tissue female 20-29	10/865	0.0021	0.71722	3.23179	19.92596	SLC9A3R2; CNTFR; TMEM37; APOL4; PCOLCE2; PPP1R1A; NAALAD2; HADH; ZNF423; LARP4	-
years GTEX-XGQ4- 2226-SM-4AT4Y adipose tissue male 50-59 years	9/722	0.00213	0.717225	3.46367	21.29601	SLC9A3R2; CNTFR; TMEM37; APOL4; PPP1R1A; ZBTB16; XYLT1; HADH; ZNF423	-
GTEX-S4Q7- 1126-SM-4AD6R breast male 20-29	9/737	0.00245	0.717225	3.38965	20.37124	CNTFR; TMEM37; APOL4; PCOLCE2; PPP1R1A; ENPP2; HADH; ELFN1; POMZP3	-
years GTEX-VUSG- 0726-SM-3GIK1 heart male 50-59 years	10/886	0.0025	0.7172	3.15085	18.87721	POPDC3; APOL4; DYRK3; DYRK2; RASL11B; PCOLCE2; FUT11; COG2; HADH; ELFN1	-

Table 7: Downstream analyses of hub genes (n = 28) associated with type 2 diabetes in different tissues of human adults: GO biological processes and KEGG pathways

Term	Overlap	P-value	Adjusted P-value	Odds Ratio	Combined Score	Genes	Regulation in T2D
Ontologies: GO biological processes							
Neutrophil degranulation (GO:0043312)	5/479	0.000481	0.061879	8.94239	68.31934	SNAP25; TNFAIP6; ARG1; MMP9; CTSC	-
Neutrophil activation involved in immune response (GO:0002283)	5/483	0.000499	0.061878	8.86574	67.39777	SNAP25; TNFAIP6; ARG1; MMP9; CTSC	-
Neutrophil mediated immunity (GO:0002446)	5/487	0.000518	0.061878	8.79036	66.49468	SNAP25; TNFAIP6; ARG1; MMP9; CTSC	-
Regulation of intrinsic apoptotic signalling pathway (GO:2001242)	2/47	0.001965	0.081572	34.06324	212.28967	MMP9; MCL1	Up
Negative regulation of intrinsic apoptotic signalling pathway (GO:2001243)	2/62	0.003393	0.081572	25.5282	145.15226	MMP9; MCL1	Up
Positive regulation of cell projection organization (GO:0031346)	2/63	0.003501	0.081572	25.10844	141.97721	STMN2; SCN1B	-
Cellular response to reactive oxygen species (GO:0034614)	2/65	0.003722	0.081572	24.30891	135.96666	DPEP1; MMP9	-
Negative regulation of apoptotic signalling pathway (GO:2001234)	2/65	0.003722	0.081572	24.30891	135.96666	MMP9; MCL1	Up
Negative regulation of cysteine-type endopeptidase activity involved in apoptotic process (GO:0043154)	2/68	0.004066	0.081572	23.20046	127.71724	DPEP1; MMP9	-
Regulation of peptide hormone secretion (GO:0090276)	2/73	0.004671	0.081572	21.56121	115.70462	SNAP25; SLC2A2	Down
Pathways: KEGG							
Insulin secretion	2/86	0.006424	0.166054	18.21245	91.9304	SNAP25; SLC2A2	Down
Apoptosis	2/143	0.016992	0.166054	10.81887	44.08705	CTSC; MCL1	-

Adrenergic signalling in cardiomyocytes	2/145	0.017442	0.166054	10.66648	43.18703	PPP1R1A; SCN1B	-
Cell adhesion molecules (CAMs)	2/145	0.017442	0.166054	10.66648	43.18703	CNTNAP2; CD226	-
JAK-STAT signalling pathway	2/162	0.021472	0.166054	9.525	36.58529	CNTFR; MCL1	Up
Transcriptional mis-regulation in cancer	2/186	0.027752	0.166054	8.27257	29.65238	ZBTB16; MMP9	Up
Arginine biosynthesis	1/21	0.029006	0.166054	36.94814	130.80559	ARG1	-
Maturity onset diabetes of the young (MODY)	1/26	0.035792	0.166054	29.55111	98.40622	SLC2A2	Down
Ascorbate and aldarate metabolism	1/27	0.037143	0.166054	28.4131	93.5634	UGT2B7	Down
Fatty acid elongation	1/27	0.037143	0.166054	28.4131	93.5634	HADH	Down

GO = gene ontology; KEGG = Kyoto Encyclopedia of Genes and Genomes; T2D = type 2 diabetes

Table 8: Downstream analyses of hub genes (n = 28) associated with type 2 diabetes in different tissues of human adults: COVID-19 related gene sets and HMDB metabolites

Term	Overlap	P-value	Adjusted P-value	Odds Ratio	Combined Score	Genes	Regulation in T2D
Diseases: COVID-19 Related Gene Sets							
SARS Perturbation Down Genes Mouse Lung from GSE19137:GPL1261:2	5/246	0.000021	0.002066	17.79812	191.63596	RPL14; DPEP1; HADH; CTSC; MCL1	-
Up-regulated by SARS-CoV-2 in NHBE from GSE147507	4/500	0.004909	0.106835	6.54435	34.79441	PPP1R15A; TAP1; MMP9; CTSC	-
Up-regulated by SARS-CoV-2 in pancreatic organoids from GSE151803	4/500	0.004909	0.106835	6.54435	34.79441	PPP1R1A; TAP1; MMP9; SCN1B	-
SARS perturbation Down Genes airway epithelium (HAE) from GSE47961:GPL6480:2	3/264	0.005837	0.106835	9.06253	46.61365	RASL11B; ENPP2; MMP9	-
SARS perturbation Up Genes PBMCs GDS1028:GPL201	3/280	0.006865	0.106835	8.53213	42.50101	PPP1R15A; ARG1; MMP9	-
SARS-CoV-2/Human Interactome Gene Set from Guzzi	2/92	0.007319	0.106835	16.99316	83.55855	ISLR; MCL1	Up
SARS Perturbation Up Genes Mouse Lung from GSE19137:GPL1261:5	3/291	0.007631	0.106835	8.20166	39.98742	ZBTB16; RPL14; MCL1	-
SARS Perturbation Up Genes Mouse Lung from GSE68820:GPL7202:4	3/357	0.013276	0.142647	6.65016	28.74044	TNFAIP6; ARG1; TAP1	-
SARS Perturbation Up Genes Mouse Lung from GSE19137:GPL1261:3	3/366	0.014189	0.142647	6.48231	27.58368	ZBTB16; RPL14; MCL1	-
Down-regulated by IAV-infection in mouse spleen	3/455	0.025142	0.142647	5.1823	19.08734	ISLR; ENPP2; SOD3	-

Miscellaneous: HMDB Metabolites							
Zinc (HMDB01303)	4/82	4.99E-06	0.000264	42.50854	518.98916	ZBTB16; SOD3; MMP9	DPEP1; -
Ethyl glucuronide (HMDB10325)	1/13	0.018053	0.035712	61.60493	247.30909	UGT2B7	Down
3-Acetoacetyl-CoA (HMDB01484)	1/13	0.018053	0.035712	61.60493	247.30909	HADH	Down
(S)-Methylmalonate semialdehyde (HMDB02217)	1/14	0.019428	0.035712	56.86324	224.09815	HADH	Down
C18H31NO14S (HMDB00632)	1/14	0.019428	0.035712	56.86324	224.09815	XYLT1	Up
Ornithine (HMDB00214)	1/15	0.020802	0.035712	52.79894	204.47351	ARG1	-
17beta-Estradiol glucuronide (HMDB10317)	1/19	0.026278	0.035712	41.05761	149.40806	UGT2B7	Down
(3alpha,5beta,20S)-20-Hydroxypregnan-3-yl beta-D-glucopyranosiduronic acid (HMDB10318)	1/19	0.026278	0.035712	41.05761	149.40806	UGT2B7	Down
3,17-Androstenediol glucuronide (HMDB10321)	1/19	0.026278	0.035712	41.05761	149.40806	UGT2B7	Down
17alpha-Estradiol-3-glucuronide (HMDB10322)	1/19	0.026278	0.035712	41.05761	149.40806	UGT2B7	Down

Table 9: Downstream analyses of hub genes (n = 28) associated with type 2 diabetes in different tissues of human adults: GTEx tissue sample gene expression profiles.

Term	Overlap	P-value	Adjusted P-value	Odds Ratio	Combined Score	Genes	Regulation in T2D
Cell Types: GTEx Tissue Sample Gene Expression Profiles Down							
GTEx-OHPK-1726-SM-48TC4 nerve female 50-59 years	4/178	0.00010	0.140263	18.9636	173.71976	SNAP25; CNTNAP2; CD226	ARG1; -
GTEx-R55E-0011-R11A-SM-2TC6I brain male 20-29 years	8/1275	0.00026	0.140263	5.905288	48.68668	TNFAIP6; ZBTB16; RPL14; TAP1; DPEP1; MMP9; CTSC; MCL1	-
GTEx-N7MS-0011-R11A-SM-2HMJS brain male 60-69 years	10/2072	0.00031	0.140263	4.82541	38.89372	ISLR; TNFAIP6; ZBTB16; XYLT1; TAP1; CD226; SOD3; MMP9; CTSC; MCL1	-
GTEx-PVOW-2726-SM-48TCA pituitary male 40-49 years	5/447	0.00035	0.140263	9.60554	76.42437	TNFAIP6; ARG1; ZBTB16; XYLT1; MCL1	-
GTEx-T6MN-0002-SM-3NMAH blood male 50-59 years	12/2999	0.00036	0.140263	4.26473	33.74814	CNTNAP2; CNTFR; ISLR; RASL11B; PPP1R1A; ZBTB16; STMN2; DPEP1; ZNF423; PHLDA1; SOD3; SCN1B	-
GTEx-PSDG-1626-SM-48TCQ breast male 50-59 years	5/487	0.00051	0.165673	8.79036	66.49468	SNAP25; CNTNAP2; RASL11B; STMN2; CD226	-
GTEx-SNOS-0526-SM-4DM54 bladder male 40-49 years	3/144	0.00104	0.232779	16.87744	115.77124	TNFAIP6; PPP1R1A; STMN2	-
GTEx-R55E-2526-SM-2TC6H brain male 20-29 years	5/570	0.00105	0.232779	7.4671	51.1853	TNFAIP6; ZBTB16; TAP1; DPEP1; CTSC	-
GTEx-TML8-0001-SM-3NMAF blood female 40-49 years	11/2914	0.00119	0.232779	3.80456	25.59278	CNTFR; ISLR; RASL11B; PPP1R1A; ARG1; ZBTB16; STMN2; ZNF423; PHLDA1; SOD3; SCN1B	-

GTEX-XYKS-0002-SM-4BRWN blood female 60-69 years	11/2979	0.001443	0.232779	3.70707	24.24791	SNAP25; CNTNAP2; CNTFR; ISLR; RASL11B; PPP1R1A; ZBTB16; STMN2; ZNF423; SOD3; SCN1B	-
Cell Types: GTEx Tissue Sample Gene Expression Profiles Up							
GTEX-XGQ4-2226-SM-4AT4Y adipose tissue male 50-59 years	6/722	0.000413	0.606044	7.33469	57.14262	CNTFR; PPP1R1A; ZBTB16; XYLT1; HADH; ZNF423	-
GTEX-XGQ4-1026-SM-4AT4L adipose tissue male 50-59 years	5/598	0.001305	0.606044	7.10426	47.18102	PPP1R15A; CNTFR; PPP1R1A; HADH; MCL1	-
GTEX-W5X1-0008-SM-4LMKA skin female 40-49 years	6/955	0.001779	0.606044	5.466902	34.61318	ISLR; RPL14; ENPP2; XYLT1; DPEP1; CTSC	-
GTEX-QV44-0008-SM-447AX skin male 50-59 years	8/1765	0.002254	0.606044	4.14684	25.2735	PPP1R15A; ISLR; RASL11B; ENPP2; XYLT1; PHLDA1; CTSC; MCL1	-
GTEX-XBED-1326-SM-4AT4F adipose tissue male 60-69 years	5/690	0.002448	0.606044	6.120913	36.80156	PPP1R15A; CNTFR; ENPP2; MMP9; MCL1	-
GTEX-R55C-1626-SM-48FEG adipose tissue male 40-49 years	5/720	0.002943	0.606044	5.854971	34.12304	CNTFR; PPP1R1A; ZBTB16; ENPP2; XYLT1	-
GTEX-XUW1-0526-SM-4BOP3 adipose tissue female 50-59 years	5/735	0.003217	0.606044	5.730196	32.88661	CNTFR; PPP1R1A; ZBTB16; ENPP2; HADH	-
GTEX-R55C-0005-SM-3GAE9 blood male 40-49 years	6/1090	0.003461	0.606044	4.752096	26.92674	TNFAIP6; ARG1; CD226; MMP9; CTSC; MCL1	-
GTEX-WH7G-1126-SM-3NMBK adipose tissue male 40-49 years	5/758	0.003672	0.606044	5.54853	31.11036	CNTFR; TNFAIP6; PPP1R1A; HADH; MCL1	-
GTEX-OIZH-0005-SM-2HMJN blood male 50-59 years	6/1105	0.003703	0.606044	4.68351	26.221	TNFAIP6; ARG1; TAP1; CD226; MMP9; MCL1	-

FIGURES

Figure 1: Stacked bar chart depicting the number of differentially expressed (up- and down-regulated) genes in the 16 datasets.

Figure 2: Venn diagram depicting the number of differentially expressed genes by the 4 tissue types.

Figure 3: Highly perturbed genes (n = 49) identified by random effects model meta-analysis in ‘MetaVolcanoR’ package with ‘metathr’ set at 0.01. Consistently upregulated genes appear in red and consistently downregulated genes appear in blue.

Figure 4: Highly perturbed genes (n = 27) identified by vote-counting approach meta-analysis in ‘MetaVolcanoR’ package with ‘metathr’ set at 0.01. Consistently upregulated genes appear in red and consistently downregulated genes appear in blue.

Figure 5: Highly perturbed genes (n = 8) identified by p-value combining approach meta-analysis in ‘MetaVolcanoR’ package with ‘metathr’ set at 0.01. Consistently upregulated genes appear in red and consistently downregulated genes appear in blue.

Figure 6: Inverse cumulative distribution of the consistently differentially expressed genes as per vote-counting approach.

Figure 7: Gene-gene interactions network visualized in GENEMANIA

Figure 8: Protein-protein interactions network visualized in STRING

Figure 9: Three different presentations of the protein-protein interactions network in Centiscape.

Figure 10: Downstream analyses of highly perturbed genes (n = 79) associated with type 2 diabetes in different tissues of human adults: (a) GO biological processes (b) KEGG pathways.

Figure 11: Downstream analyses of highly perturbed genes (n = 79) associated with type 2 diabetes in different tissues of human adults: (a) COVID-19 related gene sets (b) HMDB metabolites.

Figure 12: Downstream analyses of highly perturbed genes (n = 79) associated with type 2 diabetes in different tissues of human adults: (a) GTEx tissue sample gene expression profiles down. (b) GTEx tissue sample gene expression profiles up.

Figure 13: Downstream analyses of hub genes (n = 28) associated with type 2 diabetes in different tissues of human adults: (a) GO biological processes (b) KEGG pathways.

Figure 14: Downstream analyses of hub genes (n = 28) associated with type 2 diabetes in different tissues of human adults: (a) COVID-19 related gene sets (b) HMDB metabolites.

Figure 15: Downstream analyses of hub genes (n = 28) associated with type 2 diabetes in different tissues of human adults: (a) GTEx tissue sample gene expression profiles down. (b) GTEx tissue sample gene expression profiles up.