Transcriptional changes of tissue-specific genes in multiple endocrine organs: a study of lethal COVID-19 cases

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Abstract

Altered blood hormone and metabolite levels during and post-COVID-19 have been extensively reported. Yet, studies of gene expression at the tissue level that can help identify the causes of endocrine dysfunctions are scarce. We analyzed transcript levels of endocrine-specific genes in five endocrine organs of lethal COVID-19 cases.

Overall, 116 autoptic specimens from 77 individuals (50 COVID-19 and 27 uninfected controls) were included. All samples were tested for SARS-CoV-2 genome. Investigated organs included adrenals, pancreas, ovary, thyroid and white adipose tissue (WAT). Transcript levels of 42 endocrine-specific and 3 IFN-stimulated genes (ISGs) were measured and compared between COVID-19 cases (virus-positive and virus-negative in tissue) and uninfected controls.

ISG transcript levels were enhanced in tissues positive for SARS-CoV-2. Endocrine-specific genes (e.g., *HSD3B2*, *INS*, *IAPP*, *TSHR*, *FOXE1*, *LEP*, *CRYGD*) were deregulated in COVID-19 cases in an organ-specific manner. Transcription of organ-specific genes was suppressed in virus-positive specimens of ovary, pancreas and thyroid but enhanced in adrenals. In WAT of COVID-19 cases transcription of ISGs and leptin was enhanced independently of the presence of virus.

Our findings suggest that, in COVID-19, endocrine dysfunctions may arise especially when SARS-CoV-2 invades endocrine organs and that transcriptional alterations of endocrine-specific genes may contribute to endocrine manifestations.

Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes COVID-19, a disease presenting with a spectrum of manifestations that range from asymptomatic infection to severe pneumonia followed by multiorgan failure [1]. The reasons why severe forms of COVID-19 may occur are controversial, but evidence suggests that defects of the interferon (IFN) response and/or autoantibodies to IFNs are of prime importance [2]. Generally, it is agreed that mild COVID-19 forms associate with a prompt and robust type I IFN response that limits virus replication and spreading [3]. This response is typical of young people. Severe forms of COVID-19 are instead associated with a delayed type I IFN response that allows an extensive replication of virus and its abundant spread within the body, including endocrine organs [4–8].

Clinical studies show that a variety of endocrine functions may be altered in COVID-19, but the basis of dysfunctions is uncertain. Obesity is an important and independent risk factor for hospitalization and mortality [9]. It is known, in fact, that SARS-CoV-2 replicates in macrophages and adipocytes [7, 10] and that the adipose tissue is an important virus reservoir [11]. Obese subjects have a systemic pro-inflammatory state with abnormal production of adipokines, metabolic disorders, and hyperglycemia. In COVID-19, high glucose levels associate with a remarkable increase of acute respiratory distress syndrome that leads to mechanical ventilation and high mortality [12, 13]. Several mechanisms

contribute to hyperglycemia, including systemic inflammation with insulin resistance, administration of glucocorticoids, and the viral infection of pancreatic islets [14, 15]. The consequences of SARS-CoV-2 infection of pancreas are controversial [16–18]. Indeed, cases of new-onset diabetes following COVID-19 have been reported [19, 20], and virus-infected pancreatic islets show vascular damage and fibrosis *in vivo* [21]. However, *in vitro*, infection by SARS-CoV-2 of human pancreatic islets is noncytopathic and produces only limited cell dysfunction [16].

Glucocorticoids are produced by adrenal glands and are essential to balance the immune response [22]. In critically ill COVID-19 patients, low levels of cortisol and adrenocorticotrophic hormone (ACTH) are observed [23, 24]. Cases of central adrenal insufficiency have been reported [25, 26], and the genome and antigens of SARS-CoV-2 have been detected in adrenals at autopsy [27]. However, pathological changes of adrenals in COVID-19 are minimal [27–29], and it is debated whether adrenal insufficiency is primary or due to impairment of the hypothalamic-pituitary-adrenal (HPA) axis. COVID-19-associated transcriptional changes of pituitary genes may play a role [6].

Similarly, the hypothalamic-pituitary-thyroid (HPT) axis may be impaired in COVID-19 possibly contributing to thyroid abnormalities. Though many patients are euthyroid, a proportion of them – especially those admitted to the intensive therapy unit – manifest clinical hypothyroidism [30–32] with reduced levels of both thyroid stimulating hormone (TSH) and free thyroxine (T4) [30]. In addition, Lania et al. [33] reported thyrotoxicosis with risk of atrial fibrillation and thromboembolic events in about 20% of hospitalized COVID-19 patients.

Finally, controversial findings have been reported regarding sex hormones. While low testosterone levels associated with severe forms of COVID-19 in males [34–36], changes in sex hormones and their effects in females are not straightforward [35–37]. SARS-CoV-2 may infect ovaries [38], but sex hormone levels and ovarian functions appear not to change significantly in women [37, 39]. Li and colleagues observed temporary menstrual volume decrease or cycle prolongation in about 20% of women of child-bearing age that were recovering from COVID-19 [37].

Despite the huge number of studies addressing hormonal changes in the course of COVID-19, studies of virus-associated changes in endocrine organs remain scarce. Reports are usually limited to histopathological observations. In the present study of lethal COVID-19 cases, we evaluated the mRNA transcript levels of tissue-specific genes in five endocrine organs: adrenal gland, pancreas, ovary, thyroid, and abdominal subcutaneous white adipose tissue (WAT). Transcript levels have been compared to those of a matched control group of subjects dying abruptly of non-infectious causes. Results show that the severe forms of COVID-19 are characterized by activation of type I IFN pathways and by significant alterations of organ-specific transcripts that may be linked to endocrine dysfunctions.

Materials And Methods

Investigated cases and controls

A total of 116 autoptic organ specimens from 77 individuals were included in the study: 50 of them died of COVID-19, while 27 subjects who died abruptly of non-infectious causes (trauma, sudden cardiac death) served as controls (Table 1). Autopsies have been performed in a Biosafety Level 3 facility at the Unit of Forensic Medicine (Azienda USL Toscana Nord Ovest, Lucca, Italy) serving four major Hospitals: Pisa, Lucca, Livorno and Massa-Carrara. In COVID-19 cases (but not in controls) two post-mortem lung biopsies tested positive for the SARS-CoV-2 genome using real-time reverse transcription polymerase chain reaction (RT-PCR). The lung pathology report of COVID-19 cases confirmed the infection as the cause of death due to respiratory failure (at times accompanied by multiorgan failure). At histology, the lung parenchyma showed extensive alveolar damage, hyaline membranes, involvement of endothelial/interstitial cells, interstitial and alveolar edema, hemorrhages, microthrombi, and mononuclear cell infiltrations with macrophages as the most abundant cell type. No significant histological changes were observed in lungs of uninfected controls.

Table 1
Demographics of COVID-19 cases and controls.

	COVID-19	Uninfected controls
Age, yearsmedian (IQR)	68 (55–77)	62 (53.5–75)
Sex, malenumber (%)	36 (72.0%)	14 (51.8%)
BMI, kg/m^2median (IQR)	25.6 (23.1–29.0)	23.6 (21.7–25.0)
IQR, interquartile range; BMI, body mass index.		

Five different endocrine organs were studied: adrenal gland, pancreas, ovary, thyroid, and WAT. Some cases overlap with cases that had been investigated in earlier reports of our group [4–7]. The numbers of investigated endocrine organs of COVID-19 cases and uninfected controls are shown in Table 2.

Table 2
Endocrine tissues analyzed in the study.

Tissue	COVID-19		Uninfected Controls
	SARS-CoV-2 detected	SARS-CoV-2 not detected	
Adipose tissue	7	7	7
Adrenal gland	8	9	7
Pancreas	7	8	7
Ovary	3	9	7
Thyroid	9	13	8

RT-PCR and nCounter assay

For each case, four to six 10-µm-thick formalin-fixed paraffin-embedded (FFPE) sections were used for RNA isolation using the RNeasy FFPE kit (Qiagen, Hilden, Germany). RNA quality and quantity were assessed using an Xpose spectrophotometer (Trinean, Gentbrugge, Belgium). About 250 ng of total RNA were utilized for detection of the viral genome using the one-step Easy SARS-CoV-2 WE RT-PCR kit (Diatech Pharmacogenetics, Jesi, Italy) as previously described [6]. Briefly, the assay has a limit of detection of 5 target copies *per* reaction. Two virus targets are tested, namely the nucleocapsid (N) and the RNA-dependent RNA polymerase (RdRp) genes. A sample was considered positive when at least one of the targets was amplified at Ct values below those indicated by the manufacturer (i.e., 36th Ct for N and 38th Ct for RdRp).

Gene expression levels were measured by the nCounter system (nanoString Technologies, Seattle, WA, USA) using a custom 55-gene panel. In detail, the panel included 10 housekeeping genes used as reference (i.e., *ABCF1*, *ALAS1*, *GUSB*, *MRPS7*, *NMT1*, *NRDE2*, *OAZ1*, *PGK1*, *SDHA* and *STK11IP*); 3 IFN-stimulated genes (ISGs; i.e., *IFI44*, *OAS1* and *RSAD2*), in addition to 42 genes that were specifically expressed in the investigated endocrine organs. Endocrine-specific genes were selected based on their definition as top tissue-enriched or group-enriched in the Human Protein Atlas (https://www.proteinatlas.org). As shown in Supplementary Table S1, endocrine-specific genes were as follows: adrenal gland (n = 8) *CCN3*, *CYP11B1*, *CYP11B2*, *CYP17A1*, *CYP21A2*, *GML*, *HSD3B2*, *KCNK2*; ovary (n = 6) *CRYGD*, *HTR1A*, *KLHDC8A*, *LEFTY2*, *NXPH2*, *WFIKKN2*; pancreas (n = 11) *CPA1*, *CTRB2*, *GAD1*, *GAD2*, *GCG*, *IAPP*, *INS*, *PNLIPRP1*, *PTPRN*, *SLC30A8*, *SST*; thyroid (n = 12) *BMP8A*, *FOXE1*, *GOLGA8Q*, *ID4*, *IYD*, *PKHD1L1*, *SLC26A4*, *SLC26A7*, *TG*, *TPO*, *TSHR*, *ZNF804B*, and WAT (n = 5), *AQP7B*, *LEP*, *OR52N5*, *TM4SF19*, *TRAG1*. For the nCounter assay, about 175 ng of RNA were hybridized with probes at 65°C for 21 hours.

Data analysis and statistics

Raw mRNA transcript counts were normalized following the procedures of the Advanced Analysis module of the nSolver software v.4.0 (nanoString Technologies). Genes with an expression level above the mean plus two standard deviations of negative control probes in a proportion equal to the size of the smallest group were used from further analyses. Normalized counts were log2-transformed for downstream analyses. Principal Component Analysis (PCA) was performed using the filtered genes and following the procedures of PCAtools Bioconductor package v.2.8.0. Unsupervised clustering was carried out using heatmap3 R package v.1.1.9 and setting Euclidean and Ward as distance and clustering method, respectively. Differentially expressed genes (DEG) were computed using the best fitting model among negative binomial, simplified negative binomial and log-linear. Age, sex, and body mass index (BMI) were used in the model as confounders. Control cases were used as baseline, and two comparisons were made for each tissue type: COVID-19 cases that were virus-positive in the endocrine tissue *vs.* controls; COVID-19 cases virus-negative in the endocrine tissue *vs.* controls. The Benjamini-Hochberg method was used to adjust *P* values, and a false discovery rate (FDR) of 0.25 was considered significant. The

analyses were performed in R environment v.4.1.2 (https://www.r-project.org/, last accessed June 16, 2022), unless otherwise specified.

Results

Normalized gene transcription levels were used to evaluate the organ specificity of endocrine-related genes. As shown in Fig. 1, using the entire set of genes, the organ type was the major determinant of variation. Organ-specific clusters were observed using both Principal Component Analysis (PCA) and hierarchical clustering. The analyses did not detect any significant effects of other variables [i.e., virus detection into the tissue, sex, age, body mass index (BMI)]. Figure 2 shows that transcription of IFN-stimulated genes (ISGs, i.e., *OAS1*, *RSAD2, IFI44*) was independent on the type of endocrine organ (Fig. 2A-B) and – as expected – transcription of ISGs was activated in tissues in which SARS-CoV-2 was detected (Fig. 2B-C). Figure 2D further shows that upregulated transcription of ISGs was present in virus-positive but not in virus-negative endocrine organs. One notable exception was WAT (Fig. 2D) that showed activated transcription of ISGs independently of the detection of virus in the tissue.

Below is the analysis of transcripts levels of endocrine-specific genes *per* each investigated organ.

Adrenal gland

Seven of 8 adrenal-specific genes passed the quality checks. Only *GML* was filtered out due to low counts. PCA and hierarchical clustering showed slightly different patterns of transcription without clear distinction of COVID-19 from uninfected control cases (Fig. 3A-B). Deregulated endocrine-specific genes were observed only in virus-positive adrenal specimens, while no significant transcriptional changes were detected in virus-negative adrenals of COVID-19 cases (Fig. 3C-D). Three genes were upregulated in virus-positive adrenal tissues (Fig. 3C): hydroxy-delta-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 2 (*HSD3B2*, FC = 2.34, FDR = 0.11), cytochrome P450 family 17 subfamily A member 1 (*CYP17A1*, FC = 1, FDR = 0.15) and cytochrome P450 family 11 subfamily B member 1 (*CYP11B1*, FC = 1, FDR = 0.16).

Ovary

Six ovary-specific genes were considered for analysis. While uninfected controls showed a clustered transcription pattern, the expression profile was highly variable in COVID-19 cases (Fig. 4A-B). Interestingly, ovaries negative for SARS-CoV-2 showed no deregulation of endocrine-specific genes, whereas virus-positive ovaries showed significant deregulation of two genes (Fig. 4C-D). Transcription of crystallin gamma D (*CRYGD*) was downregulated (FC=-1.71, FDR = 0.15) while that of 5-hydroxytryptamine receptor 1A (*HTR1A*) was enhanced (FC = 1.66, FDR = 0.15). Consistent with our findings, exposure to type I IFN leads to enhanced transcription of *HTR1A* in the bovine endometrium [40].

Pancreas

After normalization, 2 genes (i.e., *GAD1* and *SLC30A8*) were filtered out because of low counts, while 9 pancreas-specific genes were considered for downstream analysis. PCA showed a degree of separation between COVID-19 and uninfected control cases on principal component 2, but some overlapping remained (Fig. 5A). Similarly, hierarchical clustering did not produce specific clustering in relation to COVID-19 diagnosis nor to virus-positivity within the organ (Fig. 5B). However, when adjusting for confounders (i.e., age, sex, BMI), transcription of 3 genes was deregulated in COVID-19 cases independently of SARS-CoV-2 detection in the tissue (Fig. 5C-D). Transcription of the pancreatic lipase related protein 1 (*PNLIPRP1*) was enhanced in COVID-19 cases *vs.* controls (FC = 0.98, FDR = 0.25 in virus-positive; FC = 1.46, FDR = 0.17 in virus-negative tissues). With regard to endocrine-specific genes, COVID-19 cases were characterized by suppressed levels of hormonal transcripts in both virus-positive and virus-negative cases compared to controls [insulin (*INS*) FC=-1.22, FDR = 0.22 and FC=-1.47, FDR = 0.17 respectively; islet amyloid polypeptide (*IAPP*) FC=-2.07, FDR = 0.11 in virus-positive and FC=-1.67, FDR = 0.21 in virus-negative cases). Of note, transcripts of the somatostatin precursor gene (*SST*) were downregulated only in virus-negative COVID-19 cases (FC=-1.6, FDR = 0.17) but not changed in virus-positive specimens.

Thyroid

Eleven out of 12 genes passed the quality check and were included for further analyses. *GOLGA8Q* was filtered out. PCA showed different patterns of transcription between COVID-19 cases and controls. Changes were particularly evident in virus-positive specimens (Fig. 6A). Differences failed to emerge in the clustering analysis, in which only a subgroup of COVID-19 cases had a distinct expression profile (Fig. 6B). In virus-positive specimens the expression of 3 genes was downregulated (Fig. 6C): zinc finger protein 804B (*ZNF804B*, FC=-1.49, FDR = 0.04), forkhead box E1 (*FOXE1*, also called Thyroid Transcription Factor 2, FC=-1.01, FDR = 0.12), and thyroid stimulating hormone receptor, the main autoantigen in Graves' disease (*TSHR*, FC=-0.73, FDR = 0.16). Interestingly, virus-positive specimens showed enhanced transcription of thyroid peroxidase (TPO), a major autoantigen in autoimmune thyroid diseases (FC = 1.18, FDR = 0.12). Finally, as compared to controls, gene transcript levels were not significantly altered in virus-negative cases of COVID-19 (Fig. 6D).

White Adipose Tissue (WAT)

Five WAT-specific genes passed quality checks. With a few exceptions, unsupervised analyses showed a separation of COVID-19 cases from controls (Fig. 7A-B). Four of 5 WAT-specific genes were significantly upregulated in COVID-19 independently of virus detection in the tissue (Fig. 7C-D). The leptin (*LEP*) gene showed the highest upregulation both in virus-positive and in virus-negative COVID-19 cases (FC = 6.07, FDR = 0.009 and FC = 6.28, FDR = 0.003, respectively). It has to be noted that leptin upregulation, typically observed in obese subjects, was independent from BMI, which has been considered as confounder. Likewise, transcription of three other genes was upregulated both in virus-positive and virus-negative specimens: trafficking regulator of GLUT4 1 (*TRARG1*; also called IFN-Induced Transmembrane Domain-Containing Protein D3, FC = 3.11, FDR = 0.02 and FC = 2.64, FDR = 0.03, respectively); transmembrane 4 L

six family member 19 (*TM4SF19*, FC = 4.25, FDR = 0.04 and FC = 3.03, FDR = 0.07, respectively); aquaporin 7B (*AQP7B*, FC = 1.57, FDR = 0.15 and FC = 1.7, FDR = 0.07, respectively).

Discussion

COVID-19 is a pulmonary and systemic disease. Though the rates of hospitalization and death have been decreasing due to vaccination and improved therapies in addition to the progressive selection of less pathogenic virus variants [41–43], the involvement of extrapulmonary organs remains a long-term threat. Among post-acute sequelae, endocrine and metabolic disorders are relatively common [44]. In spite of an increasing number of studies reporting altered blood hormones and metabolites both in the acute phase and afterwards [30, 45, 46], transcriptional alterations in endocrine organs have been barely investigated. By comparing mRNA transcript levels of genes expressed in endocrine organs of COVID-19 cases *vs.* controls, two major findings emerge: a) virus-containing tissues of five different organs show upregulation of ISGs. WAT represents an exception since transcripts of ISGs are activated both in virus-positive and in virus-negative COVID-19 cases; b) deregulated transcription of endocrine-specific genes is strictly organ-specific (Fig. 8).

Consistent with our previous studies [5–7], activation of ISGs genes is constantly observed in endocrine tissues infected by SARS-CoV-2. It is known that production of type I IFNs can be elicited in almost every cell type. Similarly, IFN receptors (IFNARs) are expressed on almost all cells, allowing them to acquire an antiviral state [47]. However, in the present study activated type I IFN responses were observed in WAT even in the absence of virus in the tissue. However, non-viral stimuli such as xenogeneic or autologous nucleic acids, activation of STING [48], autocrine signaling of IFNs that upregulates IRF7 may account for IFN activation [47]. More importantly, strong upregulation of leptin transcription has been detected in WAT of severe COVID-19 cases, independently of virus presence in the tissue. Leptin, in addition to its hormonal effects, activates lymphoid cells to produce pro-inflammatory cytokines [49], and high levels of leptin are associated with severe COVID-19 [50]. Leptin levels are also elevated in obesity, which is a major risk factor for severe COVID-19 [51]. The results show that, even after adjusting for BMI, leptin transcript levels remain considerably higher in WAT of COVID-19 cases compared to controls, showing that virus infection and/or inflammatory stimuli may induce leptin transcription.

Similarly to WAT, adrenal specimens infected by SARS-CoV-2 show a substantial upregulation of *HSD3B2*, *CYP17A1* and *CYP11B1*. These genes encode for enzymes that convert steroids to adrenal hormones. While the first two act in the synthesis of a wide range of steroids, *CYP11B1* is specifically involved in the conversion of progesterone to cortisol [52–54]. Upregulated transcription of the above genes seems not to support a primary insufficiency of adrenals in COVID-19.

An apparently controversial scenario was detected in ovaries containing SARS-CoV-2. In fact, infected ovaries exhibited downregulation of crystallin gamma D (*CRYGD*) and upregulation of the 5-hydroxytryptamine (serotonin) receptor 1A (*HTR1A*) gene. Crystallin gamma D has a very similar sequence and structure to that of crystallin beta, but it is monomeric [55]. While there are no clear

attributed functions of gamma-crystallin in ovaries, beta-crystallin does influence female fertility by regulating apoptosis in granulosa cells and follicular atresia [55]. Hence, downregulation of *CRYGD* may be consistent with alterations of the menstrual cycle observed in women recovering from COVID-19 [37]. In addition, the enhanced expression of serotonin receptor 1A is in line with decreased serotonin serum levels observed in severe COVID-19 cases [56]. Similar to what found in humans, in mice the Zika virus also targets the ovaries inducing a type I IFN response that is associated with disordered steroidogenesis [57].

In thyroid, alterations of gene transcription were selectively observed when SARS-CoV-2 was present in the tissue. Changes affected factors associated with thyroid dysfunction [58]. Transcription of *ZNF804B* and *FOXE1* was downregulated; ZNF804B is possibly associated with antiviral defense [59], while *FOXE1* promotes the expression of multiple thyroid-specific genes, including those encoding for thyroglobulin, thyroid peroxidase, thyroid dual oxidase 2, pendrin and other transporters [60–63]. *TSHR* gene was also downregulated. Since serum *TSH* levels are generally low in mild to severe forms of COVID-19 [64], low levels of the TSH receptor indicate a possible impairment of the HPT axis. In a scenario of suppressed function, the enhanced expression of thyroid peroxidase may appear not justified. However, non-endocrine regulatory mechanisms may be operative during microbial stress responses [65, 66]. Indeed, thyroid peroxidase is a major autoantigen in thyroid autoimmunity and a key player against oxidative stress [67].

Finally, the exocrine and endocrine pancreatic tissue deserves a separate discussion. First, in COVID-19 cases, the pancreas is the only tissue for which alterations of gene transcription are seen in the absence of activated IFN responses and independently of the viral presence in the tissue. Second, the expression of pancreatic lipase-related protein 1 (*PNLIPRP1*) was enhanced. Differently from its paralogs (pancreatic triacylglycerol lipase and *PNLIPRP2*), *PNLIPRP1* lacks lipolytic activity and also inhibits pancreatic lipase [68]; thus, enhancement of a lipase inhibitor may be part of a defensive response. In addition, suppression of lipid catabolism may be linked to a switch of infected cells to glycolytic metabolism [69]. Third, and more important, the results show the downregulation of two beta cell genes that code for insulin (*INS*) and islet amyloid polypeptide (*IAPP*). Both hormones are crucial in the regulation of blood glucose levels, and are frequently downregulated in diabetes. The findings support a potential failure of beta cells in COVID-19 and reminds that stress associated with a reduction of intracellular proinsulin may activate inflammatory pathways in beta-cells [70]. Unexpectedly, somatostatin (*SST*) mRNA transcripts were downregulated only in virus-negative pancreas specimens. Somatostatin is produced by pancreatic delta cells and regulates pituitary growth hormone, thyroid stimulating hormone, and most hormones of the gastrointestinal tract [71]. Contextualization of somatostatin downregulation in COVID-19 needs further attention.

Our study has some limitations. First, the sample size *per* group in each investigated endocrine organ is relatively small, though more than a hundred tissues were analyzed. Second, the significance level was set at FDR = 0.25, a way to identify significant features in relatively small size groups. On the other hand, important sources of variation such as age, sex and BMI have also been considered in the analyses.

Finally, only a few genes highly specific for each organ were evaluated. Their functional relevance, however, does indicate alterations of interest for translational medicine.

In conclusion, transcriptional alterations in endocrine organs of individuals who died because of COVID-19 are tissue-specific. In most organs, significant changes were observed only when the SARS-CoV-2 genome was present in tissue. While infected ovary and thyroid showed downregulation of tissue-specific genes, in adrenals transcription of endocrine genes was enhanced, possibly as part of the stress response. Notably, in beta cells hormone genes were suppressed independently of the presence of virus in the tissue. This is reminiscent of type 1 diabetes where beta cell functions are inhibited in an inflammatory context ([66]). Also in WAT, the enhanced transcription of functional genes is independent of virus presence in tissue and may be linked to the inflammatory context. Our findings provide evidence that endocrine dysfunction may arise in COVID-19, especially when the virus invades endocrine organs. Clinicians should be aware that endocrine manifestations in the acute phase of COVID-19 and in the post-COVID syndrome may derive from transcriptional changes of endocrine-specific genes.

Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author contributions

AMP, FB and AT designed research study; AMP, DB, EM, SN, AB and FS acquired data; AMP analyzed data; FB and AT funding acquisition; AMP, FB and AT wrote the manuscript; all authors have read, revised and approved the final version of the manuscript.

Data availability

The datasets generated during the current study are available from the corresponding author on reasonable request.

Ethics approval

The study has been approved by the local Ethics Committee (Comitato Etico Area Vasta Nord-Ovest, Italy; protocol number 17327; May 14, 2020). The procedures employed in the study are in accordance with the ethical standards of the Local Ethics Committee and with the 1964 Helsinki Declaration and its later amendments.

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Figures

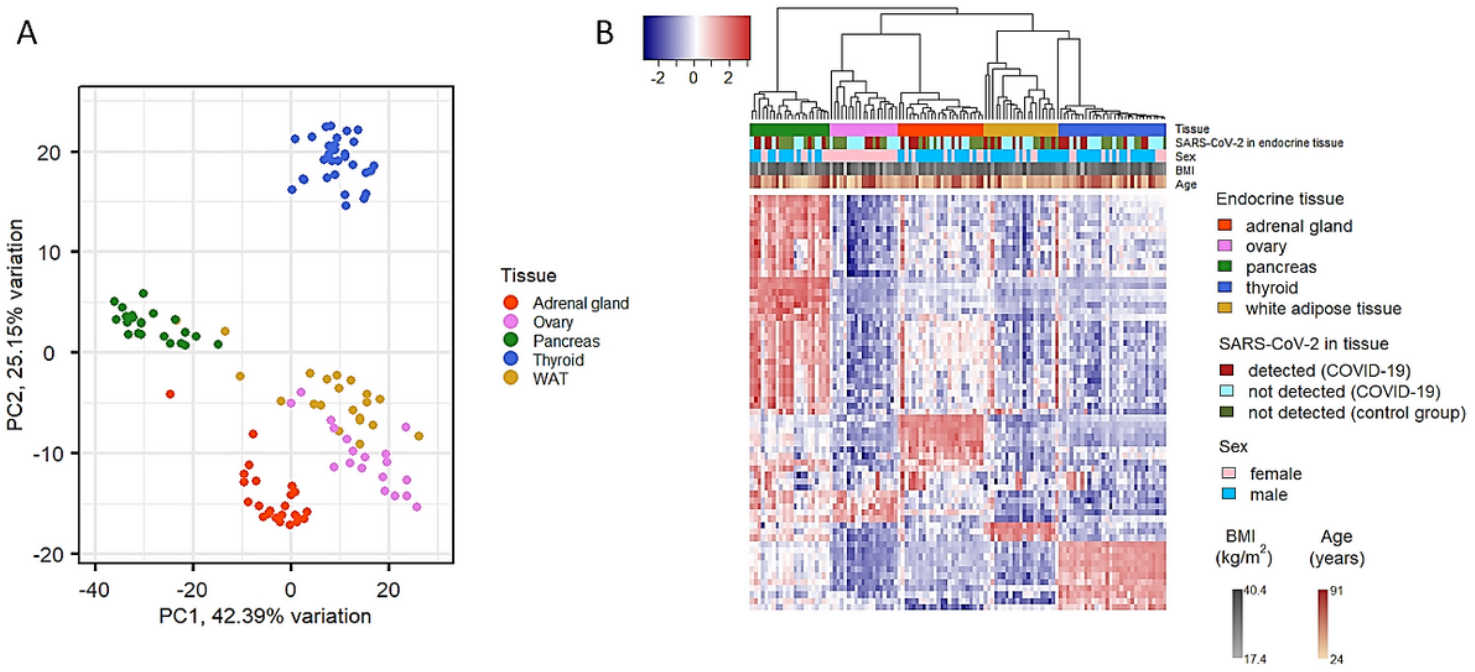

Figure 1

Organ-specific expression of endocrine genes. Transcript levels of endocrine genes were expressed in an organ-specific manner. A) Principal components 1 and 2 that account for the majority of variation were plotted, and a neat separation of samples according to organ type can be observed. Results were confirmed by unsupervised clustering (B); five different clusters specific for each organ type were produced. No effect of age, sex, BMI and detection/absence of SARS-CoV-2 could be observed at this level

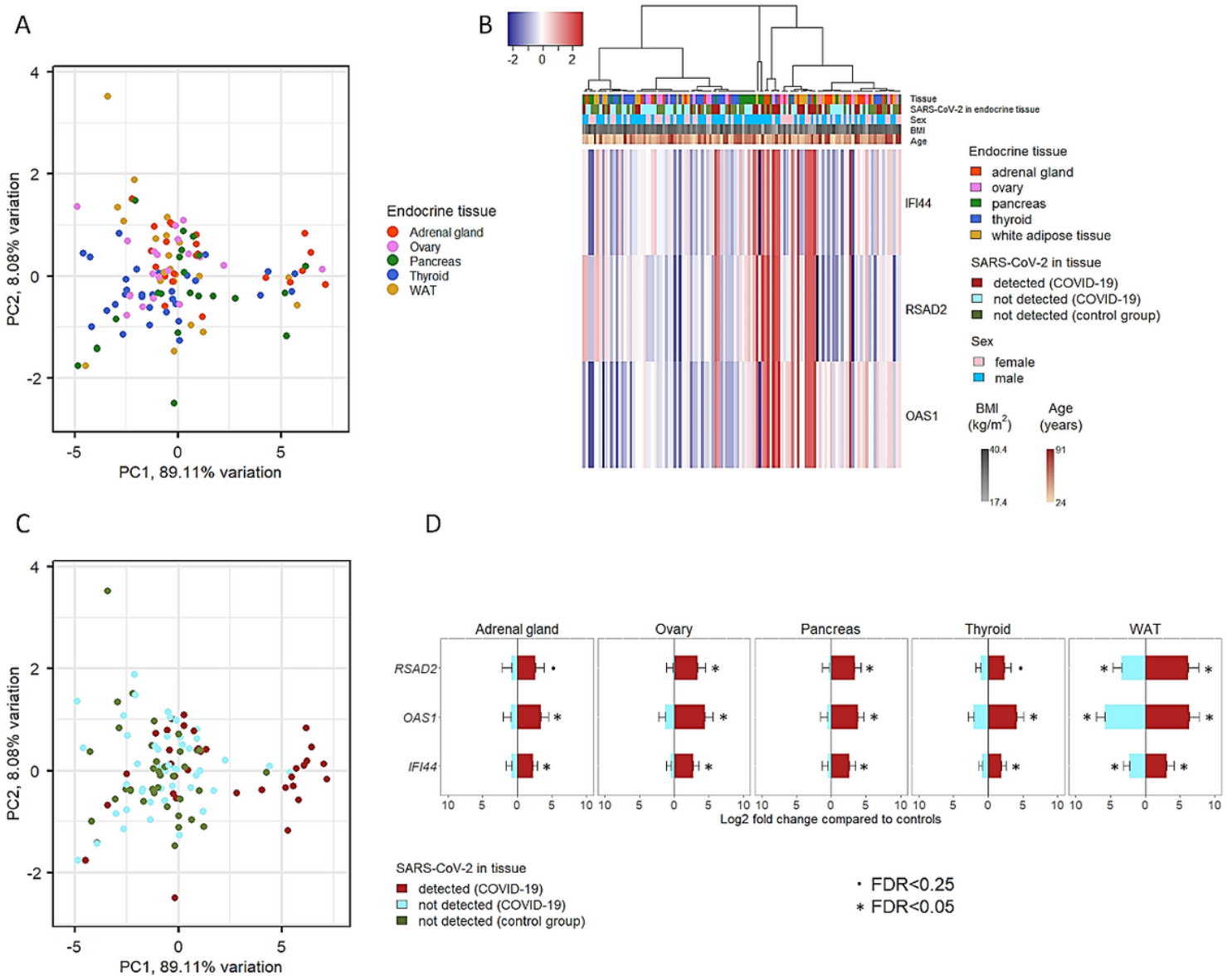

Figure 2

Expression of IFN-stimulated genes (ISGs). For ISGs, PCA showed no effect of organ type (A). Similarly, no organ-specific clusters were produced by hierarchical clustering (B). A cluster enriched in specimens positive for SARS-CoV-2 genome can be observed. This is confirmed on PCA (C), where a group of SARS-CoV-2-positive samples (in red) shows a peculiar expression pattern. Panel D shows the log2 fold change of ISGs (i.e., *RSAD2*, *OAS1* and *IFI44*) in virus-positive (red) and virus-negative (cyan) COVID-19 samples compared to the baseline of controls. In all organ types, ISGs are significantly upregulated only when the virus could be detected in the tissue. The only exception is the WAT, where enhanced transcription of ISGs is observed also in virus-negative COVID-19 specimens

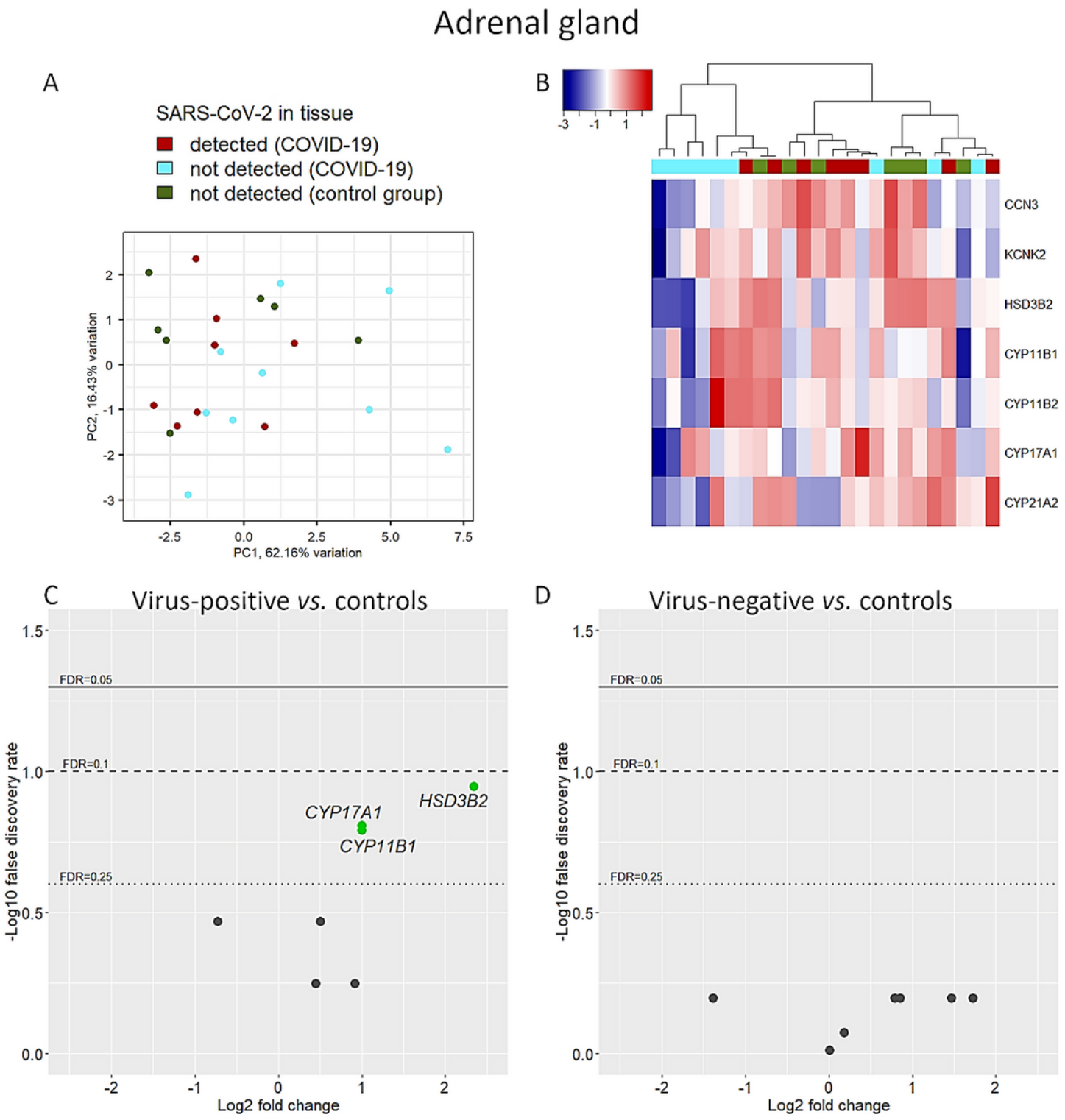

Figure 3

Adrenal-specific genes. Unadjusted transcript levels of adrenal genes did not produce specific clusters on PCA (A) nor on clustering analysis (B). However, when adjusting for confounders, 3 genes (i.e., *HSD3B2*, *CYP17A1* and *CYP11B1*) were significantly upregulated in SARS-CoV-2-positive adrenals (C), while no differences were observed in virus-negative COVID-19 specimens compared to controls (D)

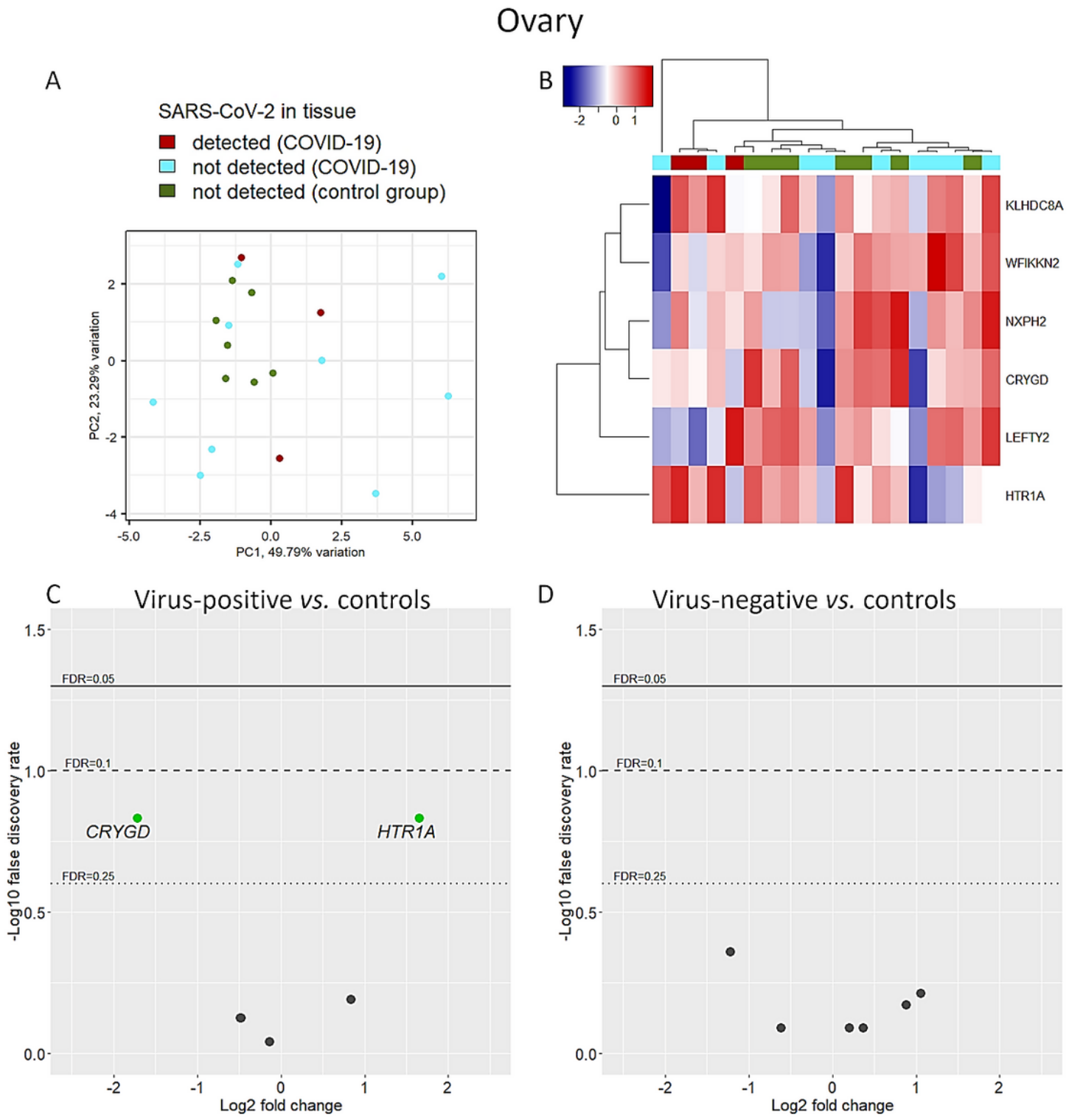

Figure 4

Ovary-specific genes. Ovary-specific genes were heterogeneously expressed in COVID-19 samples (A and B). However, in virus-positive tissues *CRYGD*transcripts were significantly suppressed, while *HTR1A* transcript levels were enhanced (C). The SARS-CoV-2-negative COVID-19 cohort did not show deregulations of any gene (D)

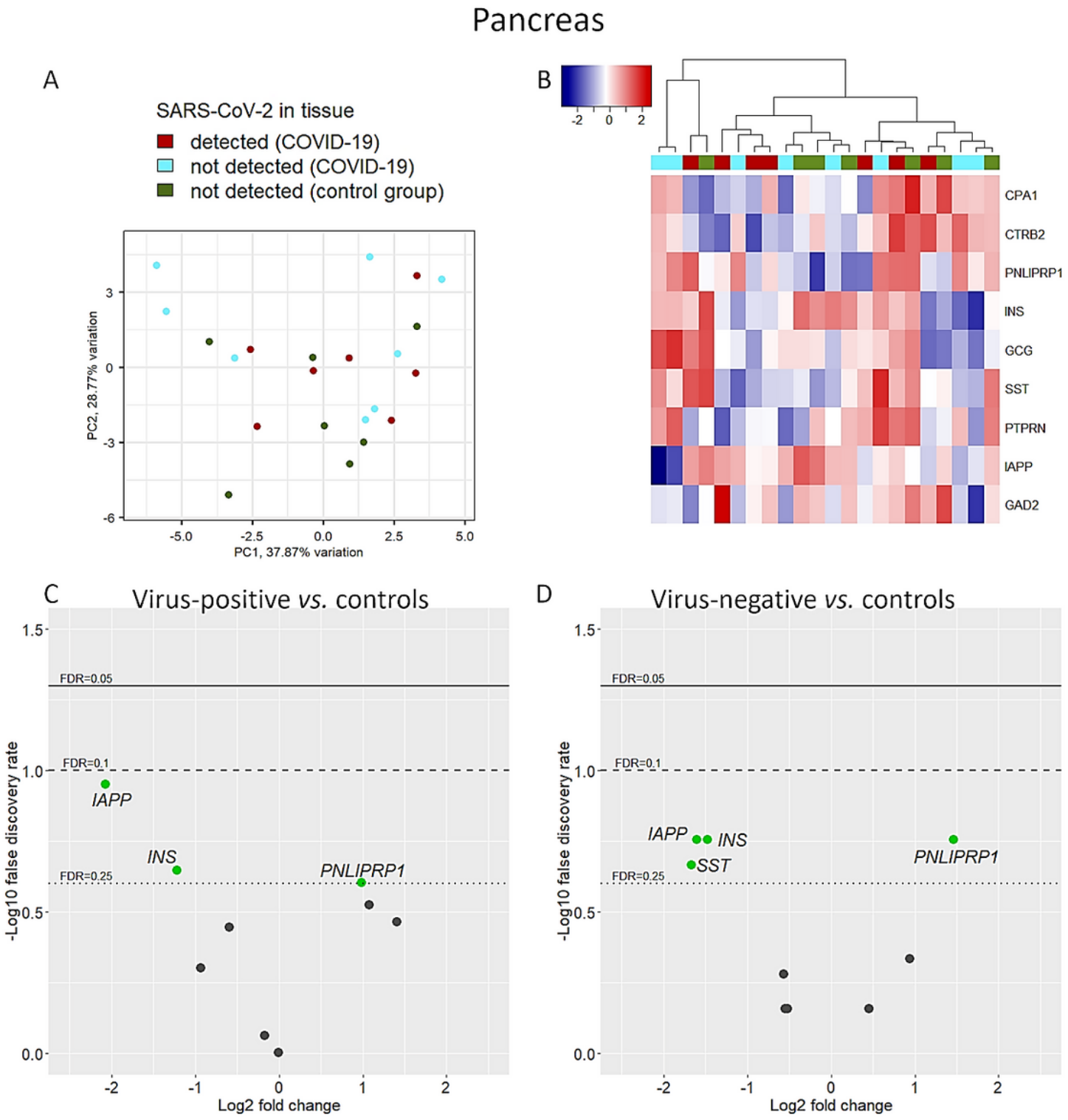

Figure 5

Pancreas-specific genes. Like in the majority of organs, unadjusted level of specific transcripts did not clearly separate pancreatic specimens according to COVID-19 or virus positivity (A and B). Differential expression analysis, however, produced 2 downregulated (i.e., *IAPP* and *INS*) and 1 upregulated (*PNLIPRP1*) genes in virus-infected tissues (C) and in COVID-19 specimens in which the virus was not

detected. Notably, somatostatin (*SST*) transcript levels were downregulated in the virus-negative COVID-19 group (D)

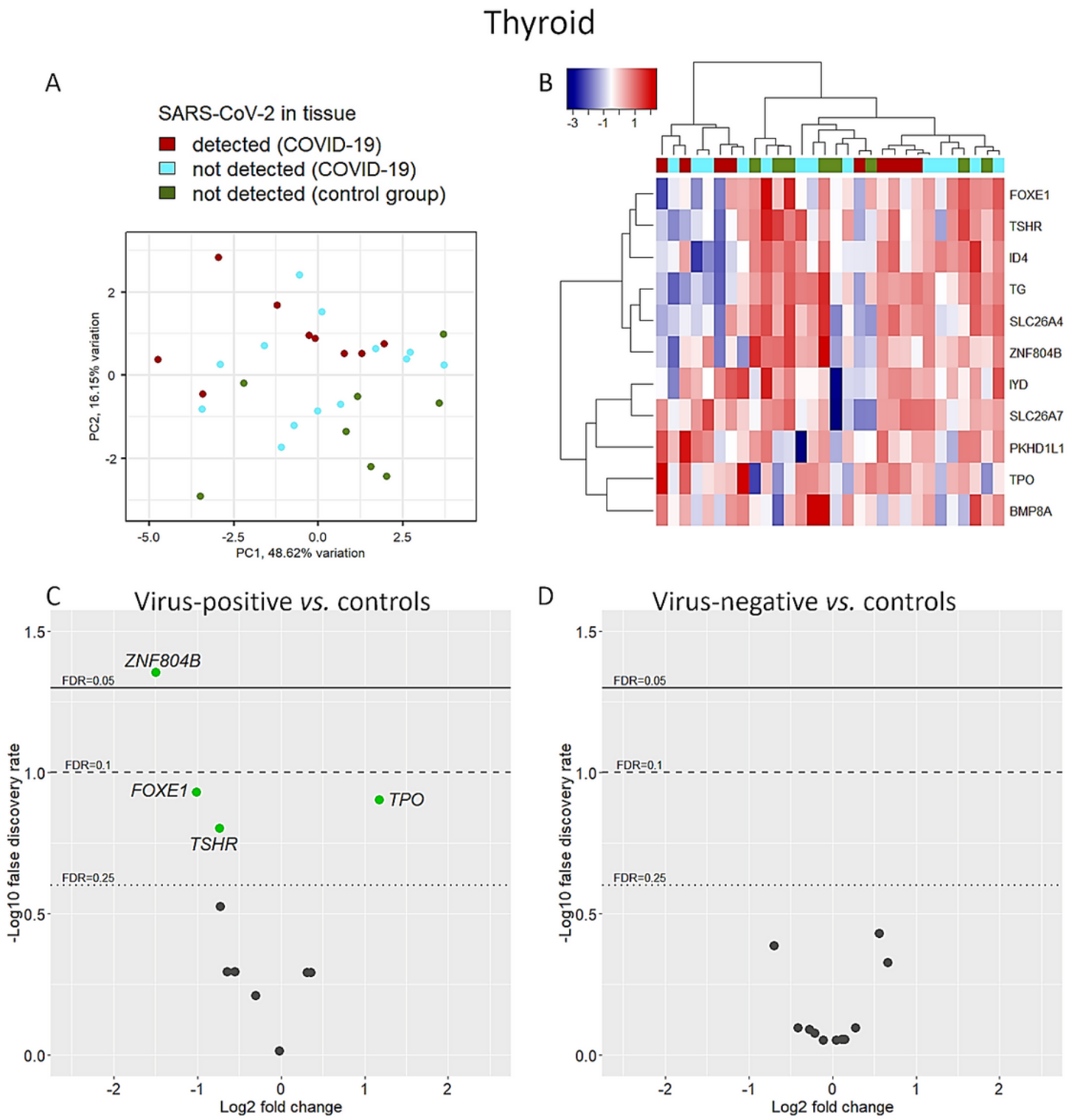

Figure 6

Thyroid-specific genes. Unlike other organs, distinct patterns of expression between COVID-19 and controls were observed in thyroid specimens using unadjusted thyroid-specific transcript levels (A and B).

In virus-positive samples, *TPO* transcription was enhanced compared to controls, while mRNA transcript levels of *ZNF804B*, *FOXE1* and *TSHR* were downregulated (C). No gene deregulation was observed in COVID-19 cases that were negative for SARS-CoV-2 in tissue (D)

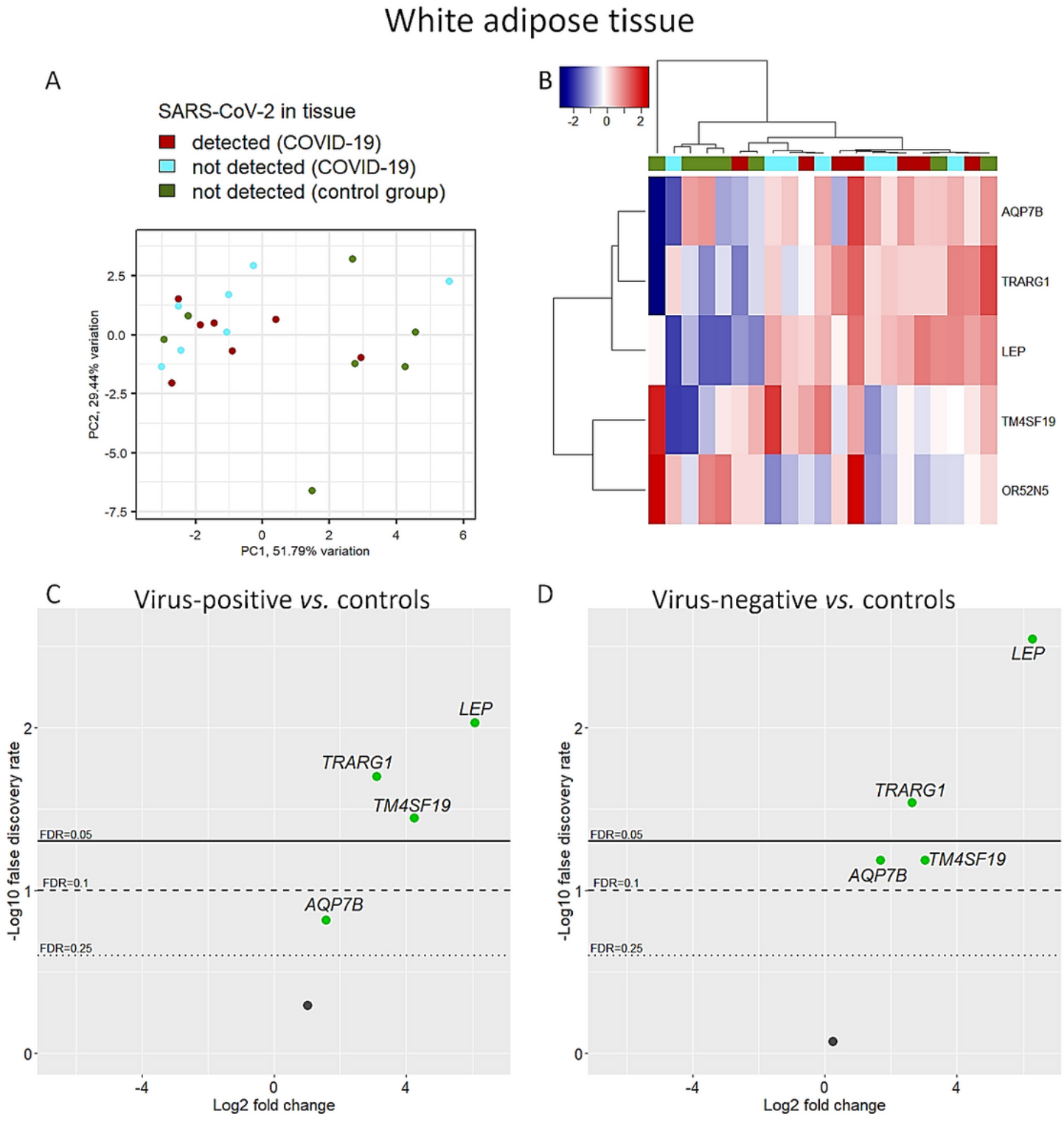

Figure 7

WAT-specific genes. Some differences between COVID-19 and control cases were observed both by PCA (A) and hierarchical clustering (B). With regard to differentially expressed genes, both virus-positive (C) and virus-negative (D) COVID-19 cases showed upregulation of 4 genes compared to the control group, especially the leptin (*LEP*) gene, accompanied by the *TRARG1*, *TM4SF19* and *AQP7B* genes

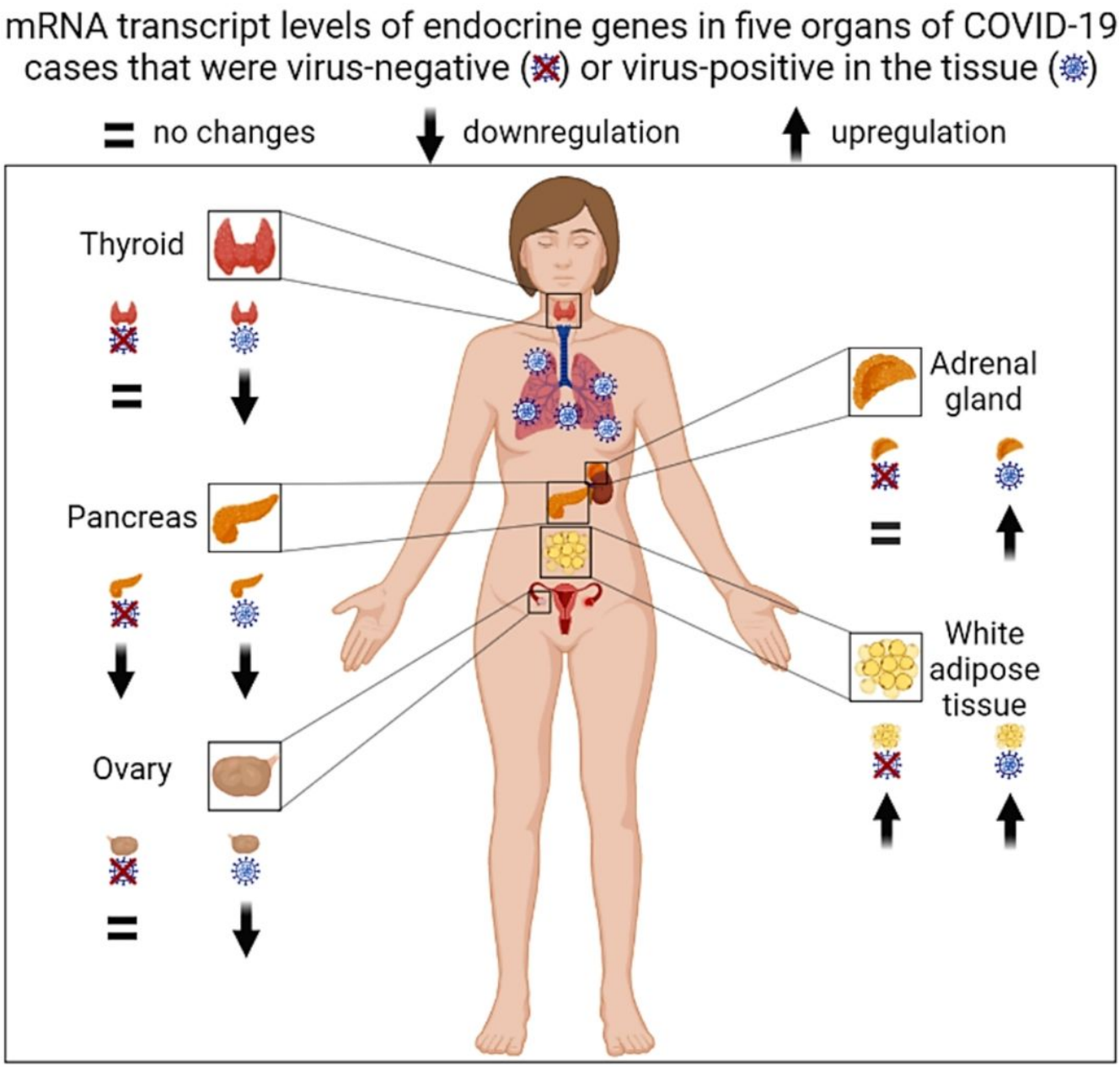

Figure 8

Summary of the study. Transcriptional deregulations in endocrine tissues from lethal COVID-19 cases are organ-specific. In thyroid, pancreas and ovary endocrine-specific genes are downregulated, while in adrenals and WAT they are upregulated. Deregulations occur when SARS-CoV-2 directly invades thyroid,

ovary and adrenal gland; however, mRNA transcript changes in pancreas and WAT are independent of virus infection of the organ. Created with BioRender (https://biorender.com/)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Keymessages.docx
- SupplTableS1.xlsx