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ORIGINAL ARTICLE

Association of overexpression of PLD6, CHRAC1 and PDCD5 with type 2 diabetes mellitus

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ABSTRACT

Aim Diabetes type 2 (DT2) is a metabolic disease characterized by high blood sugar caused by insulin resistance and/or insufficient insulin production. The pathogenesis of DT2 is complicated by both genetic predisposition and environmental and lifestyle variables. At least 150 genetic variants have been linked to the probability of having DT2. The aim of this study was to determine the expression of PLD6, CHRAC1, and PDCD5 genes in type 2 diabetic patients.

Methods Information on 12 DT2 patients was obtained from the Gene Expression Omnibus (GEO) using the series identification (ID) (GSE34008). The analysis tools GEO2R, String Utils (STRING), University of ALabama at Birmingham Cancer data analysis (UALCAN), and the Cancer Genome Atlas (TCGA) were used. The human protein atlas provided details on gene cancer.

Results Only ten genes with expression differences ranging from low to high were selected. PLD6, CHRAC1, and PDCD5 were detected to have higher expression in patients compared to controls. The number of patients with primary pancreatic adenocarcinoma for SLC16A4, DERK2, and CHRAC1 was greater than that of healthy controls. Concerning the severity of cancer, all chosen genes demonstrated a greater proportion of affected individuals compared to the control group.

Conclusion There are multiple genes whose increased expression is linked to type 2 diabetes.

Keywords: β -cells, diabetes, gene, GEO

INTRODUCTION

Diabetes type 2 has become a major public health concern (1,2). Previously regarded as a problem solely affecting Western people, the disease is quickly reaching worldwide significance. Important roles in defining DT2 risk are played by lifestyle and behavioural factors. In humans, a combination of low birth weight and rapid growth during childhood has been linked to reduced glucose tolerance in maturity (3,4). The islets of Langerhans play a vital role in the development of DT2. Under normal circumstances, an increase in blood sugar levels following a meal causes the pancreas to release insulin. Several genes are related to DT2 on specific chromosomes, as proven by genome-wide linkage analyses (5,6). Numerous genes are active in β-cells or may be involved in insulin production, which lends credence to the theory that β-cell dysfunction is a crucial final step on the path to diabetes (7). Few genes appear to be involved in insulin sensitivity, and genes involved in the insulin signalling pathway show infrequently in DT2 genome-wide association studies (GWAS) analyses. Genes discovered through genome-wide association research affect the size or function of β -cells (8,9). Discoveries suggest that epigenetic mechanisms are likely involved in DT2 as a crucial interface between the genetic and environmental effects. Epigenetic modifications are reversible changes that occur without modifying the DNA sequence (10,11).

According to the findings of some studies, there are at least 150 genetic variants that can be related to the likelihood of having DT2. The majority of these alterations are common and can be found in both persons with and

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without diabetes. Every individual has some variations that increase the risk and others that decrease the danger. The combination of these alterations influences a person's proclivity to develop the disease (12).

The age group between 35- 60 years old has the highest heritability estimates for DT2. GWAS have been crucial in advancing our understanding of the genetic architecture of type 2 diabetes within the past decade. According to one study, those who carry risk mutations in DT2 susceptibility genes are unable to alter their insulin secretion in response to lower insulin sensitivity (13,14).

Now, new processes in the pathophysiology of diseases like DT2 are being uncovered, and the genetic landscape of DT2 is becoming clearer. Recent advancements in systematic and unbiased large-scale genotyping and sequencing approaches undoubtedly open the door for future discoveries and unprecedented revelations (15).

Phospholipase D family member 6 (*PLD6*) is a member of the phospholipase D family of mitochondrial enzymes. It changes cardiolipin on the cytoplasmic surface of mitochondria into phosphatidic acid, which causes mitochondria to join together. The most malignant tissues have moderate cytoplasmic positivity for *PDL6* expression. A few cases of thyroid, carcinoid, prostatic, and pancreatic cancers showed high activity (16,17). Programmed cell death 5 (PDCD5) (TF-1) is a protein that contains 125 amino acid residues. Apoptosis-related gene PDCD5 was cloned from human leukaemia cells in 1999. PDCD5 protein was expressed in many cell lines, suggesting it regulates pathological and physiological processes. Breast, astrocytic glioma, hepatocellular carcinoma, gastric cancer, and chronic myelogenous leukaemia had a decreased PDCD5 expression (18). CHRAC1 (YCL1) is a histone-fold protein that interacts with other histone-fold proteins to bind DNA in a sequence-independent way. These histone-fold protein dimers are responsible for the generation of bigger enzymatic complexes, which are then used for DNA transcription, replication, and packing (19,20).

The aim of this study was to identify genes linked to DT2 by assessing their expression levels with microarrays.

PATIENTS AND METHODS

Patients and study design

Pancreatic islets of Langerhans were isolated from the pancreas of two groups: group 1 included 12 DT2 patients and group 2 included 12 healthy donors. The information was obtained from the Gene Expression Omnibus (GEO) using the series identification (ID) (GSE34008) (16).

Methods

The Microarray gene expression kit was used to analyse all of the samples (Affymetrix GeneChip, Santa Clara, CA, USA). A total of 1900 genes were analysed using bioinformatics tools. We performed a broad scan of microarray-identified genes in this study. Many bioinformatics techniques were utilized to discover genes that have a direct influence on the onset of DT2. The level of expression for the genes that have a direct effect on the condition had to be measured as well. GEO2R was used to analyse gene expression.

StringUtils (STRING) tool was used to detect the protein-protein interaction. Using the ALabama at Birmingham CANcer data analysis (UALCAN) server, the heat map of the specified genes was generated.

Individuals diagnosed with DT2 have their genes experimented on pancreatic adenocarcinoma (PAAD). Several genes were examined, and it was shown that the relative expression levels of each gene across the primary tumour according to the Cancer Genome Atlas (TCGA) analysis samples.

The STRING database includes information on both experimental and computational prediction approaches. The algorithm uses functional genomic data to accurately evaluate the degree of interaction between proteins. By evaluating the data, it is able to construct effective interaction maps that are vital in understanding the progression of the illness as a result of interactions between proteins.

The UALCAN tool examines cancer-related data. It generates the necessary data and charts after identifying the genes that cause cancer. The unique data is obtained from patients who underwent genetic analysis. The program analyses clinical protein consortium data, which includes complete proteomics. It also analyses the gene and protein expression in juvenile brain tumours.

The (TCGA) initiative identifies the molecular composition of over 20,000 genes from primary cancer cases and then compares them to normal samples from 33 confirmed cancer types.

Statistical analysis

Volcano diagram showed differentially expressed genes (-log10 p-value) vs the magnitude of change (log2 fold change). The limma (Venn diagram) approach was used to examine and download gene overlap between several comparisons. The genes in each Venn diagram region can be downloaded by selecting the relevant contrasts. The values of the selected samples were plotted using a box plot. Samples were randomly assigned to the DT2 and control groups and were each given a different colour. The width of the distribution can be used to judge the suitability of the chosen samples for differential expression analysis. The density plot was used to estimate the sample distribution values based on the colour group. It supplements the boxplot for verifying data normalization before differential expression analysis. The limma (qqt) plot compares the dataset's quantiles

with the theoretical quantiles of the t-test distribution. This graph helps evaluate the quality of the limma test outcomes.

The moderated t statistic has proven highly beneficial in microarray research. The statistic was expressed as d/(s + s0), where d is the difference in means between two groups, s represents the estimate's pooled standard deviation, and s0 is a tiny constant. Test results are often discrete, with thousands of genes near to background levels and integer densities less than plus or minus 30.

When the statistical analysis was complete, the application displays the results of a comparison of 250 genes with the lowest possible p values, grouped in ascending order. The final statistics table includes the following data:

The p-value after accounting for numerous tests (column with adj. p. in Table 1) numbers included the fundamental statistics from which the findings may be evaluated. Genes with lower adjusted p-values will be the most dependable and tightly connected. Results with adjusted p-values <0.05 were picked, which is similar to setting the false discovery rate (FDR) to 0.05 and allowing 5% of discovered genes to be false positive. GEO2R analysis shows 250 genes with the lowest p-values.

The original p-value before multiple testing adjustment established the findings' deviance plus or minus the standardmean. The shrunken t-test was used for comparison of particular groups. The B statistics represents log probabilities that the gene will be expressed differently than other genes.

The logarithm was employed in base 2 because it is simple to understand. Doubling the initial scale is equivalent to altering the log2 times by one. Doubling it four times is equivalent to increasing the log2 times by two. As a consequence, the scale is doubled according to the change in expression.

An evaluation of gene expression data was made examining gene set enrichment within the cluster using differential functional annotation. Depending on whether the differentially expressed genes were associated with a specific biological activity or have molecular roles within the cell, the data were shown as curves or an Iimma plot.

The findings were presented in the form of a table that provides a ranking of 20 genes with the lowest p-values.

RESULTS

Following the completion of an expression screen for each gene, a total of 10 genes were chosen because of the differences in gene expression that were identified between them (Table 1), as well as the difference in gene expression between the two patient groups (Figure 1).

By plotting statistical significance (the adjusted p value and the spot identifier), a volcano diagram showed differentially expressed 10 genes in 12 samples (-log10 p value) vs magnitude of change (log2 fold change) (Supplemental Digital Content 1).

Figure 2A depicts a normal expression as the black dot genes of 27578 of the total probs. The variation in gene expression was not substantially different across the patient groups (Figure 2B).

The outcomes of the study demonstrated that no genes were linked to each other among the patient groups (Figure 2C).

Patients' values clustered around the median suggest that the data are standardized and mutually comparable and that the value distributions across the various samples are consistent. Figure 2D shows the results of applying a log transformation and a normalization to the data. There was no difference in the distribution of density curves between the groups (Figure 2).

Figure 3A illustrates the data after log transformation and normalization have been completed. After the comparison of the dataset's quintiles with the theoretical quintiles of the t-test distribution, the ideal alignment of the dots along a straight line indicated that the values for the moderated t-statistic generated from the test corresponded to their theoretically predicted distribution (Figure 3B).

StringUtils (STRING) tool was used to detect the protein-protein interaction. Analyses of protein-protein interactions found that only two out of 10 genes had a binding association (SYT12 and NFKB1). These two genes have down-regulation in DT2 (Figure 4).

Individuals diagnosed with DT2 have their genes experimented on pancreatic adenocarcinoma (PAAD). Several genes were examined, and it was shown that their expression was different in DT2 patients compared to controls. The relative expression levels of each gene across the primary tumour were extracted according to The Cancer Genome Atlas (TCGA) analysis data. Analysing the findings reveals a difference in the expression of chosen genes between normal persons and people with PAAD in their four grades. *PLD6* and *PDCD5* expression levels were similar in early grades of the illness, although the fourth grade of PAAD was expressed at a higher rate. *SYT12*, *KCNMB2*, *HTRA3*, and *DYRK2* were all similarly expressed in early grades, but their expression declined by the fourth grade. In contrast, gene expression of *SYT12*, *PLD6*, and *NFKB1* declined below that of normal persons in the first three grades. *SLC16A4*, *KCNMB2*, and *HTRA3* gene expression was essentially negligible during the fourth grade of infection (data are not shown). The proteins of interest, *PLD6, PDCD5*, and *CHRAC1* were all found to be expressed in DT2 in our study.

Table 1. Ten selected genes with different expression and their positions

D	ad. p	p	t	B	logFC	RANGE_STRAND	GB_ACC	ORF	Gene symbol
cg02880176	1	0.0036902	-3.22	-3.355	-0.06841	-	NM_178836.2	201164	*PLD6*
cg12724357	1	0.004158	3.17	-3.47	0.03241	+	NM_177963.2	91683	*SYT12*
cg12002314	1	0.0047481	-3.12	-3.598	-0.00902	+	NM_017444.3	54108	*CHRAC1*
cg06310844	1	0.0102633	-2.79	-4.333	-0.02533	+	NM_004708.2	9141	*PDCD5*
cg08812936	1	0.0104801	2.78	-4.352	0.06312		NM_005832.3	10242	*KCNMB2*
cg01137708	1	0.0155268	2.61	-4.722	0.006	+	NM_003998.2	4790	*NFKB1*
cg24105933	1	0.0158808	-2.6	-4.743	-0.00819	+	NM_053044.2	94031	*HTRA3*
cg09494546	1	0.0144363	-2.64	-4.654	-0.02018		NM_004696.1	9122	*SLC16A4*
cg22621695	1	0.0003305	-4.2	-0.991	-0.03026	+	NM_003583.2	8445	*DYRK2*
cg21522988	1	0.0077983	2.91	-4.072	0.02299		NM_018099.3	55711	*FART2*

D, identification profile number; adj. p, p-value after correction for multiple testing; t, t-statistic of the shrunken t-test; B, B-statistic or log-odds that the gene differentially expressed; logF C, log2-fold change between the two experimental conditions; RANGE_STRAND, gene sequence forward + or -; GB_ACC, the gene identification number in GenBank; ORF, open reading frame

DISCUSSION

The development of genome-wide association studies, which look for single nucleotide polymorphisms (SNPs) that are more prevalent in individuals with a given condition, has added an intriguing new angle to DT2 genetics research (21,22). While several investigations on possible genes and linkages had been done before the early 2000s, only a few susceptibility loci had been pinpointed (21). More than 60 SNPs were associated with DT2 by 2016 (22). Gene-wide association studies (GWAS) have looked at how changes in genes like ABCC8 and KCNJ11 affect the earliest oral drug sulfonylureas, and genes like SLC12A1 and AQP2 affect thiazolidinediones (TZDs) (23). All of these things help doctors decide for their patients what doses of medication to use for DT2 prevention, treatment, and management. Other SNPs and genes related to insulin signalling, including KCNJ11 and PPARG, as well as those implicated in drug metabolism, including CYP2C9, have been discovered by researchers (24,25).

Recent research has revealed that a number of epigenetic mechanisms and variables increase the risk of developing DT2 due to their essential function in numerous cellular processes. Although the GLP1 receptor and paired box 4 (PAX4) are two of the main genes that are linked to β-cell development and functioning, epigenetic methods also affect gene networks that are implicated in insulin resistance and insufficiency (26,27).

When studying the effects of new environmental exposures, it would be very helpful to include parents of both people with and without diabetes in future studies. Last but not least, a better understanding of the genetic structure of DT2 at the molecular level will help classify the different types of diabetes into subtypes. This will lead to a more precise medical treatment based on the underlying pathophysiology (28,29). Among the genes with different levels of expression, one stands out. This gene is linked to several complications of DT2. Information that came out of this work's integrative approach could be used to improve other kinds of therapy. The fact that DT2 patients and normal people have different expressions of genes linked to DT2 and its complications shows that the conditions for these complications to happen are already present in DT2. These observations show that there is already a change in the way protective genes are expressed in DT2 conditions. Obesity is by far the most important risk factor that interacts with genes to cause complications from DT2, followed to varying degrees by inflammation, diet, and stress (30,31).

Better prediction capacity is anticipated as a result of increased data availability and refined statistical approaches for evaluating interactions between genes and the environment. Researchers are gaining vital new insights into the pathophysiology of DT2 as it becomes clear that these genes play a crucial role in glucose control. It is hoped that they will lead to more effective means of diagnosis and treatment in the future (32,33). Future applications may include, but are not limited to, more accurate risk assessment, the identification of novel pharmaceutical targets, and the development of more narrowly targeted therapeutics. One example is the use of gene therapy or epigenetic reprogramming to change diabetes susceptibility (34,35).

A survey of the expression of about 1900 genes in type 2 diabetes patients was done for the present study. Only 10 genes with varying expression differences between low and high were chosen. The examined genes *PLD6*, *PDCD5*, and *CHRAC1* were found to have a greater expression in patients compared to controls. We found no significant differences in the expression of any gene across any of the study samples because every one of them fell within the permitted limit. Using a density plot analysis based on the t-test distribution supported the degree of expressive sequences.

Figure 1. Expression levels of the 10 investigated profile genes vary considerably between the control and patients with diabetes mellitus type 2 (DT2)

SYT12 and *NAFKB1* were found to be closely linked through protein-protein interaction only in the targeted loci. Heat map analysis of gene expression revealed that *PDCD5* and *HTRA3* both show elevated expression that is significantly higher than the border close to the genes as an indicator of the tumour phenotype.

The number of primary pancreatic adenocarcinoma patients was greater than that of healthy controls for *SLC16A4*, *DERK2*, and *CHRAC1*. Concerning the severity of cancer, all selected genes revealed a greater proportion of affected individuals compared to the healthy control group. The number of patients with grade 4 pancreatic adenocarcinoma was significantly higher than the number of patients with other grades for the genes *PLD6*, *PDCD5*, and *CHRAC1*.

When monitoring the expression of the three genes under study in various types of cancer, the following expression levels were observed: *PLD6* expression was

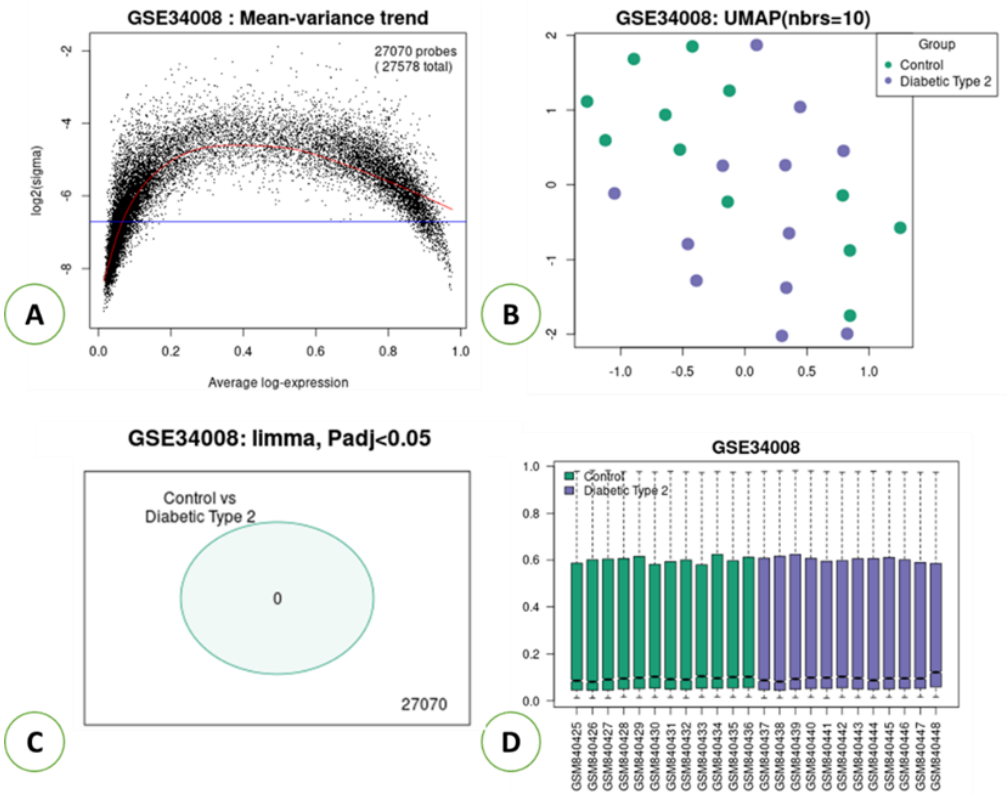

Figure 2. A) Volcano plot of gene frequency, where black dots represent normal gene expression; B) the UMAP plot depicts the distribution of samples according to their respective colors; C) the Limma plot for the groups of patients indicates no correlations between groups with p adj. <0.05; D) the box plot of the patient's profile is standardized and interchangeable

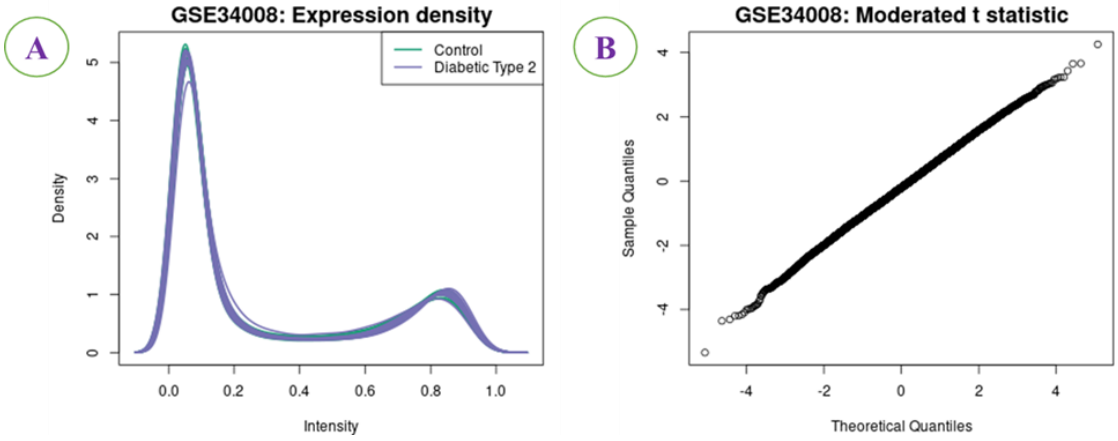

Figure 3. A) Density plot: sample distribution separated into two groups based on color; B) limma (qqt): the layout of the dataset per the theoretical quantiles of the t-test distribution

found to be elevated in patients with thyroid, carcinoid, pancreatic, and prostate malignancies. *PDCD5* was found to be elevated in pancreatic cancer and head and neck cancer patients. *CHRAC1* increased considerably in patients with leukaemia and lymphoma, more so than in patients with other tumours. High expression of *PLD6*, *PDCD5* and *CHRAC1* is not only deemed a marker for cancer patients but also for patients with type 2 diabetes, according to the findings of the current study. To fully understand how epigenetics play a part in DT2 and other chronic diseases, more human studies are required. This is because the observed and determined heritability may be caused by several epigenetic mechanisms and factors, such as intrauterine life as seen in animal studies, rather than inherited variations in DNA sequence. There are multiple genes whose increased expression is linked to type 2 diabetes. *PLD6*, *PDCD5* and *CHRAC1* are regarded to be DT2 markers. These genes are highly expressed in the largest proportion of grade 4 pancreatic cancer patients compared to other cancer grades.

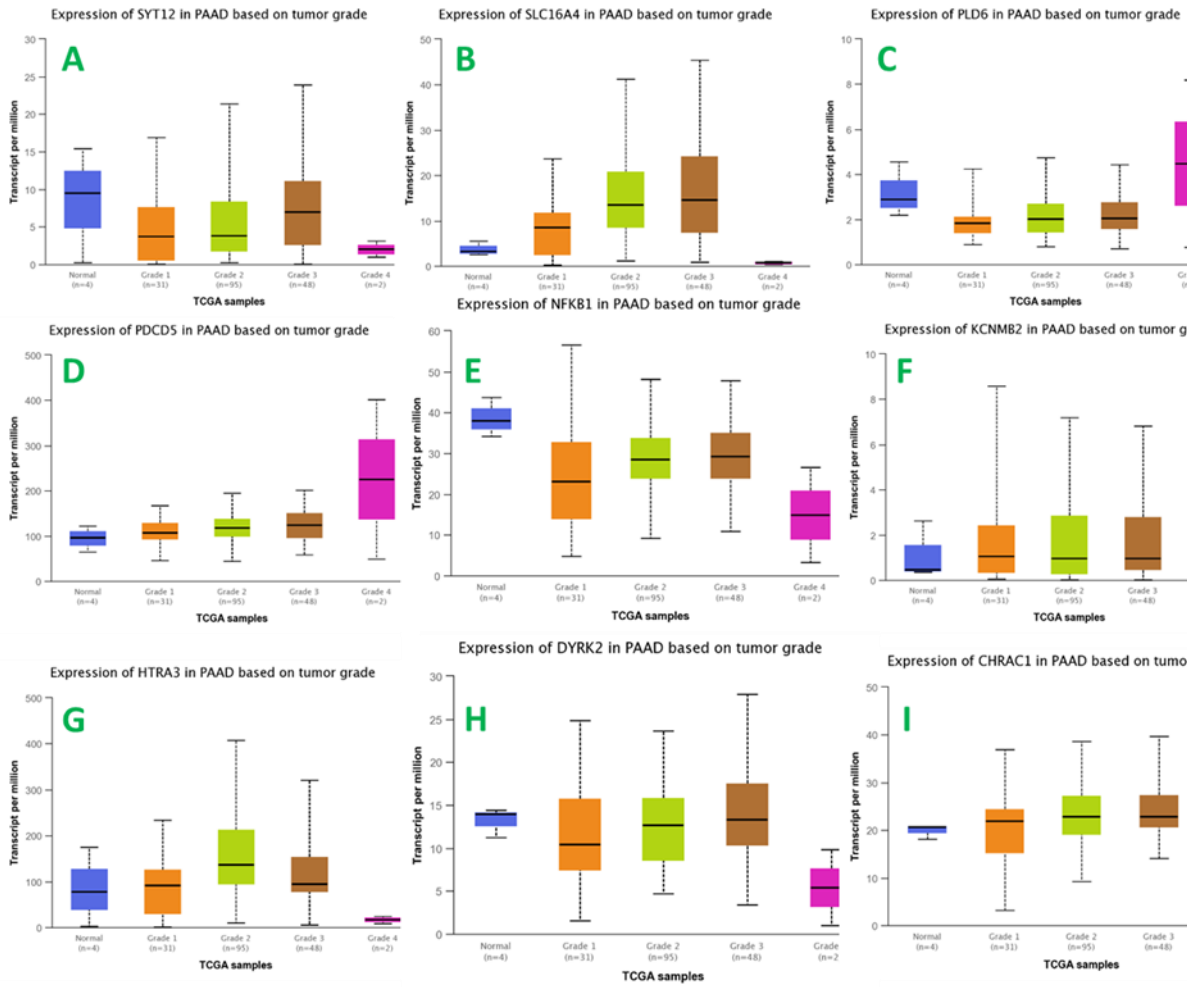

Figure 4. Tumor grade of selected genes in PAAD compared with the normal tissues. A) SYT12; B) SLC16A4; C) PLD6; D) PDCD5; E) NFKB1; F) KCNMB2; G) HTRA3; H) DYRK2; I) CHRAC1

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TRANSPARENCY DECLARATION

Conflict of interests: None to declare.

SUPPLEMENTAL DIGITAL CONTENT (SDC)

Supplemental Digital Content is avaible on the link: https://doi.org/10.17392/1739-21-02.

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