PDE12 in COVID-19 and in Type 1 Diabetes

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Article

Keywords:

Posted Date: April 14th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1524929/v1

Abstract

Type 1 diabetes (T1D) incidence is increased after COVID-19 infection in children under 18 years of age. Interferon-α-activated oligoadenylate synthetase and downstream RNAseL activation degrade pathogen RNA, but can also damage host RNA when RNAseL activity is poorly regulated. One such regulator is PDE12 which degrades 2'-5' oligoadenylate units, thereby decreasing RNAseL activity. We analyzed *PDE12* expression in islets from healthy control subjects, individuals with newly (median disease duration 35 days) and recently (5 years) diagnosed T1D, and individuals with type 2 diabetes (T2D). We also analyzed *PDE12* single-nucleotide polymorphisms (SNPs) relative to T1D incidence. *PDE12* expression was decreased in individuals with recently diagnosed T1D, in three of five individuals with newly diagnosed T1D, but not in individuals with T2D. Two rare *PDE12* SNPs were found to have odds ratios of 1.80 and 1.74 for T1D development. Decreased *PDE12* expression after COVID-19 infection may explain the up to 2.5-fold increase in T1D incidence.

Introduction

Recent research has shown that the incidence of type 1 diabetes (T1D) is increased up to 2.5-fold after coronavirus disease 2019 (COVID-19) infection in children under 18 years of age[1,2]. Similar increases in new-onset T1D have also been reported in adults[3]. One theory that explains how viral infections may lead to T1D involves the interferon (IFN)-α-activated latent ribonuclease (RNAseL) signaling pathway[4]. When IFN-α mediated cell stimulation induces downstream activation of 2'-5' oligoadenylate synthetases (OASs), the high levels of 2′-5' oligoadenylate (2-5A) produced bind to and activate RNaseL. Excessive RNaseL activity may lead to the degradation of both pathogen and host RNA, thereby causing cellular damage[5,6]. This activity is regulated by phosphodiesterases such as PDE12, which degrade 2-5A molecules, suppressing RNaseL activation. In fact, a direct link between PDE12 and OAS has been described in a PDE12-null HeLa cell line[7]. PDE12-null cells were also resistant to infection with encephalomyocarditis virus, human rhinovirus and respiratory syncytial virus, highlighting a protective effect that is associated with decreased PDE12 activity and thereby increased RNaseL activity. In addition, a separate study on inflammatory pathways in patients with T1D found that PDE12 levels are decreased in the peripheral blood of individuals with new-onset T1D (i.e., mean diabetes duration of 0.22 years)[8].

Results

From the Affymetrix analysis (Fig. 1), we observed significant decreases in *PDE12* expression for the islets of individuals with recently diagnosed T1D (median disease duration, 5.0 years) and for islets from biopsies originating from donors with recurrent T1D after pancreas transplantation. *PDE12* expression had decreased in autoantibody-positive individuals, but not significantly. Furthermore, three of the five individuals with newly diagnosed T1D (median disease duration, 35 days) exhibited low levels of *PDE12*

expression. However, *PDE12* expression was not altered in individuals with type 2 diabetes (median disease duration, 2.0 years).

The single-nucleotide polymorphism (SNP) analysis revealed that individuals with the two rare *PDE12* SNP variants shown in Table 1 had an odds ratio of 1.80 and 1.74 for developing T1D.

Table 1
SNPs close to *PDE12* that were associated with type 1 diabetes

Position	Allele	MAF	dbSNP	p-value	OR	Consequence
3:57.547.247	T/C	0.001	rs143375472	1.77e-6	1.80	3-UTR variant
3:57.562.439	G/T	0.0005	rs536228505	0.00053	1.74	Intron variant

SNPs close to the *PDE12* gene that were associated with the development of T1D were identified at two positions within the human genome. Abbreviations: PDE12, phosphodiesterase 12; MAF, minor allele frequency; SNP, single-nucleotide polymorphism; T1D, type 1 diabetes; UTR, untranslated region.

Discussion

The observed decrease in *PDE12* expression seems to have a protective effect against viral infections because it upregulates RNaseL activity in beta cells and other cells[7]; however, it may have the unfortunate side effect of triggering beta-cell damage and subsequent diabetes pathogenesis. Vaccines against COVID-19 should not activate the RNaseL cascade and therefore should not increase the incidence of T1D. Prolonged RNaseL activity may damage and kill cells[9]. Therefore, RNaseL activity must be carefully regulated to protect against viruses without compromising cellular function. Consequently, any treatments that inhibit PDE12 activity and thereby stimulate antiviral defenses should only be given for short durations, to prevent damage to cells. In fact, we found that *PDE12* expression levels are decreased in individuals with recently diagnosed T1D (median disease duration, 5.0 years). During viral infection, which may initiate T1D development, individuals have high levels of PDE12 activity which makes combating the virus difficult. In the post-virus phase there is a decrease in *PDE12* expression, leading to beta cell damage. Here, stimulating *PDE12* expression might have inhibited T1D development. Thus, if COVID-19 infection is responsible for decreasing PDE12 levels after infection, the pathological setting for developing diabetes might be established.

This link between COVID-19 and T1D supports the theory that viruses can act as pathogenic triggers for T1D. Recent research has shown that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) decreases insulin expression and induces transdifferentiation of beta cells from COVID-19-infected and deceased donors[10,11]. Furthermore, beta cells readily express the angiotensin converting enzyme 2 receptor[12] used by SARS-CoV-2 for host entry, and βTC3 cells and isolated rat beta cells show substantially higher 2-5A activity upon IFN-α stimulation when compared to αTC3 cells or rat alpha cells[13]. These observations may explain why beta cells are at increased risk of RNaseL-mediated cellular damage upon viral challenge, even though the virus itself is not toxic. Together, these data may explain

the increased incidence of T1D after COVID-19 infection and provide valuable insight into the pathogenesis of T1D.

Methods

Human tissue: Pancreatic tissue from donors was collected in the Diabetes Virus Detection (DiViD)[14] and Network for Pancreatic Organ Donors with Diabetes (nPOD)[15] studies, with informed consent obtained from all participants. Briefly, DiViD donors with diabetes had a surgical resection of the pancreatic tail, between three and nine weeks after their type 1 diabetes diagnosis, while nPOD material originates from cadaveric organ donors. The procedures were approved by The Norwegian Government's Regional Ethics Committee (reference 2009/1907); nPOD donors with approval by the University of Tennessee Health Science Center (UTHSC) local Institutional Review Board [reference 10-00848-XM]). All experiments were performed in accordance with relevant guidelines and regulations.

Microdissection of pancreatic islets: Acquired pancreatic samples were laser microdissected as described previously[16]. Briefly, frozen tissue sections from nPOD and DiViD was microdissected with the Arcturus Pixcell II laser capture microdissection system (Arcturus Bioscience, Mountain View, CA, USA). Islets from 2–5 sections per donor were detected by autofluorescence and pooled together, and afterwards subjected to RNA extraction with the Arcturus PicoPure RNA Isolation Kit (Applied Biosystems, Grand Island, NY, USA). RNA quality and quantity was validated with the Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA), and samples underwent gene expression analysis with the Affymetrix expression arrays (Thermo Fisher, Santa Clara, CA, USA) as described previously[17].

SNP analysis: Genotyping data were retrieved from the UCSD T1D GWAS meta-analysis[18] and similarly for T2D[19].

Statistics: *PDE12* expression statistics were calculated using Welch's *t*-test and visualized with R software (ver. 4.1.2; R Development Core Team, 2021) using the tidyverse (ver. 1.3.1), ggplot2 (ver. 3.3.5), and ggpubr (ver. 0.4.0) packages.

Declarations

Author contributions: KB conceptualized the project and together with HT and KJ wrote the original manuscript draft. LK, KDJ, and IG provided the analyzed material and performed the RNA expression analysis. FP performed the SNP analysis. All authors edited, reviewed, and approved the final manuscript.

Data availability: Donor data is not publicly available due to patient protection rules by the ethical committees, but anonymized PDE12 expression data is available upon request. The protocols used can be obtained upon request to the corresponding author. Researchers interested in acquiring biological sample from the donors can apply through the DiViD and nPOD programs.

Code availability: The code used to produce visuals and statistics for Fig. 1 can be obtained upon request from the corresponding author.

Competing interests: The authors declare no competing interests.

Ethics committee approval: DiViD and nPOD studies were approved by The Norwegian government's regional ethics committee (reference 2009/1907) and by the University of Tennessee Health Science Center's local institutional review board [reference 10-00848-XM]).

Funding: The study is funded by the Axius and Bagger Sørensen foundations. The funding sources had no role in conceiving this study or preparing the manuscript.

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Figures

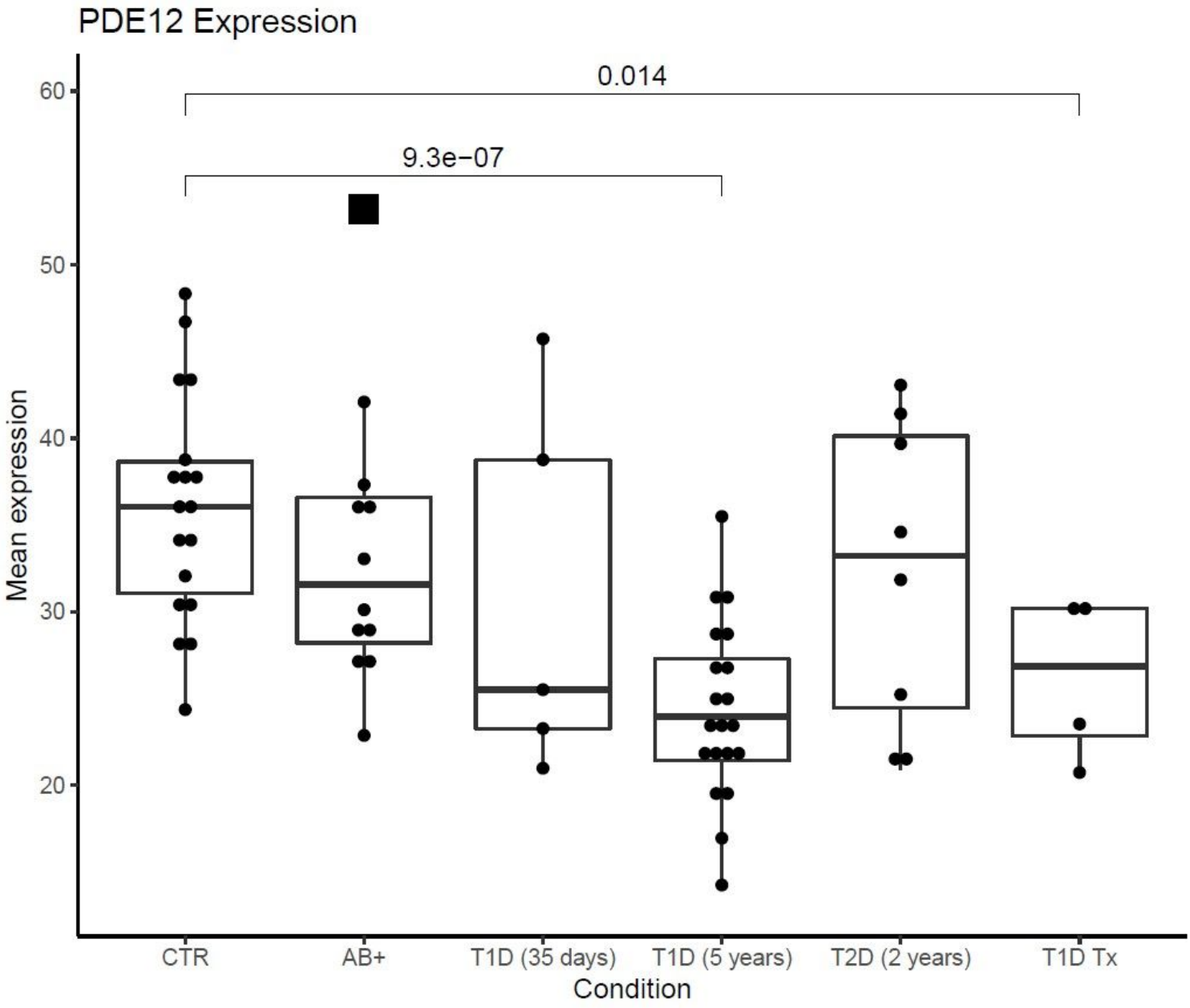

Figure 1

Phosphodiesterase 12 (*PDE12*) gene expression.

CTR: non-diabetic controls (*n* = 18); AB+: non-diabetic autoimmune antibody-positive donors (*n* = 12); T1D (median disease duration, 35 days): donors with newly diagnosed type 1 diabetes (*n* = 5); T1D (median 5 years): donors with recently diagnosed type 1 diabetes (*n* = 20); T2D (median 2 years): donors with type 2 diabetes (*n* = 8,); T1D Tx: biopsies from donors with recurrent T1D (*n* = 4). Boxes indicate 25% and 75% quartiles, whiskers 1.5× interquartile ranges, and squares mark outliers. The *p*-values shown were calculated using unpaired two-sided *t*-tests relative to CTR. Test statistics for CTR vs T1D (5 years): t-statistic 6.054, 95%CI 7.74;15.59, degrees of freedom 31.997, mean of CTR 35.95, mean of T1D (5 years) 24.29. Test statistics for CTR vs T1D Tx: t-statistic 3.43, degrees of freedom 5.87, 95%CI 2.78;16.81, mean of T1D Tx 26.16.