PDE12 in type 1 diabetes

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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. One theory that explains how viral infections may lead to T1D involves interferon (IFN)-α-activated latent ribonuclease (RNAseL) signaling. When IFN-α mediates 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. 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. 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).

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 was also 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) (Table 1).

The single-nucleotide polymorphism (SNP) analysis revealed that individuals with the two rare PDE12 SNPs showed in Table 2 had an odds ratio of 1.80 and 1.74 for developing T1D.

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; 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. 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 35 days).
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. Then, in the post-virus phase there is a decrease in PDE12 expression which leads to beta-cell damage. Here, stimulating PDE12 expression might have inhibited T1D development.

The 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. Furthermore, beta cells readily express the angiotensin converting enzyme 2 (ACE2) receptor 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. 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 might support the increased incidence of T1D after COVID-19 infection and provide valuable insight into the pathogenesis of T1D. However, several other mechanisms for the comorbidity has been suggested including the ACE2-receptor and pro-inflammatory cytokine changes. Since our study is fairly small, it is not possible at this point to have a firm conclusion of the relationship between COVID-19 and T1D. However, the PDE12 hypothesis seems not to be in conflict with the other mechanisms just mentioned.

Methods

Human tissue. Pancreatic tissue from donors was collected in the Diabetes Virus Detection (DiViD) and Network for Pancreatic Organ Donors with Diabetes (nPOD) 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 (see Table 1). 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. 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 to 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.
| Clinical diagnosis | Age | Biological Sex | BMI (kg/m²) | Duration of diabetes (years) | C-peptide (nmol/L) | Hb1Ac (%) | Peak glucose (mg/dL) |
|--------------------|-----|----------------|-------------|-----------------------------|-------------------|-----------|---------------------|
| No diabetes        | 65  | Male           | 24.2        | 0                           | 2.8               | 0         | 212                 |
| No diabetes        | 21  | Male           | 27.8        | 0                           | 3.52              | 0         | 0                   |
| No diabetes        | 30  | Male           | 20.6        | 0                           | 17.91             | 0         | 279                 |
| No diabetes        | 16  | Male           | 14.9        | 0                           | 2.94              | 0         | 211                 |
| No diabetes        | 68  | Female         | 23.7        | 0                           | 2.97              | 0         | 208                 |
| No diabetes        | 14.2| Male           | 30          | 0                           | 5.37              | 0         | 249                 |
| No diabetes        | 38  | Male           | 21.7        | 0                           | 11.1              | 0         | 183                 |
| No diabetes        | 22.7| Male           | 28.9        | 0                           | 7.61              | 0         | 312                 |
| No diabetes        | 51  | Male           | 25.2        | 0                           | 0.00              | 6.2       | 336                 |
| No diabetes        | 17  | Female         | 26.4        | 0                           | 2.75              | 0         | 1039                |
| No diabetes        | 42.9| Female         | 23.4        | 0                           | 0.51              | 5.2       | 0                   |
| No diabetes        | 45.8| Female         | 25          | 0                           | 4.45              | 5.6       | 256                 |
| No diabetes        | 45.1| Female         | 35.1        | 0                           | 0.55              | 6.1       | 292                 |
| No diabetes        | 31  | Female         | 26.9        | 0                           | 6.23              | 5.5       | 221                 |
| No diabetes        | 33  | Female         | 29.5        | 0                           | 1.92              | 5.3       | 153                 |
| No diabetes        | 47  | Female         | 19.7        | 0                           | 0.00              | 0         | 177                 |
| No diabetes        | 21.8| Female         | 20.7        | 0                           | 2.74              | 0         | 167                 |
| No diabetes        | 42  | Male           | 31          | 0                           | 0.47              | 5.6       | 298                 |
| T1D                | 22.6| Female         | 21.6        | 7                           | <0.05             | 0         | 494                 |
| T1D                | 14.2| Male           | 26.3        | 4                           | <0.05             | 0         | 425                 |
| T1D                | 31.2| Male           | 27          | 5                           | <0.05             | 0         | 526                 |
| T1D                | 27.1| Male           | 25.9        | 11                          | <0.05             | 0         | 363                 |
| T1D                | 21  | Female         | 22.8        | 1.5                         | <0.05             | 0         | 1499                |
| T1D                | 13  | Male           | 21.3        | 5                           | 0.42              | 13.1      | 645                 |
| T1D                | 13  | Male           | 17.4        | 0                           | 0.1               | 13.3      | 664                 |
| T1D                | 5   | Female         | 11.95       | 0.25                        | 0.1               | 0         | 587                 |
| T1D                | 37.2| Female         | 30.9        | 20                          | 0.2               | 0         | 630                 |
| T1D                | 18.8| Female         | 25.2        | 8                           | <0.05             | 0         | 1105                |
| T1D                | 22.9| Male           | 28.8        | 7                           | 0.00              | 0         | 256                 |
| T1D                | 19.2| Male           | 23.7        | 5                           | <0.05             | 0         | 509                 |
| T1D                | 12  | Male           | 20.3        | 1                           | 0.18              | 0         | 480                 |
| T1D                | 12  | Female         | 26.6        | 3                           | 0.05              | 9.8       | 310                 |
| T1D                | 11  | Male           | 12.9        | 8                           | 0.06              | 0         | 824                 |
| T1D                | 26  | Female         | 26.6        | 15                          | 0.48              | 0         | 860                 |
| T1D                | 24  | Female         | 24.4        | 4                           | <0.05             | 10.5      | 615                 |
| T1D                | 13.1| Female         | 24.8        | 1.58                        | <0.05             | 0         | 248                 |
| T1D                | 12  | Female         | 22          | 9                           | <0.05             | 8.9       | 641                 |
| T1D                | 43.5| Male           | 28.7        | 21                          | <0.05             | 0         | 0                   |
| AB +               | 69.2| Female         | 21.3        | 0                           | 1.84              | 0         | 226                 |
| AB +               | 23.2| Female         | 17.6        | 0                           | 2.01              | 5.4       | 267                 |
| AB +               | 40.3| Male           | 29.7        | 0                           | 0.51              | 5.6       | 449                 |
| AB +               | 37  | Male           | 26.3        | 0                           | 5.43              | 0         | 185                 |
| AB +               | 4.3 | Female         | 14.8        | 0                           | 8.95              | 0         | 342                 |
| AB +               | 41.4| Male           | 27.4        | 0                           | 13.55             | 0         | 0                   |
| AB +               | 64.8| Male           | 34.3        | 0                           | 26.18             | 0         | 0                   |
| AB +               | 48.5| Female         | 24.5        | 0                           | <0.05             | 0         | 440                 |
| AB +               | 40  | Male           | 19.8        | 0                           | 13.34             | 0         | 259                 |
| AB +               | 31.9| Male           | 21.9        | 0                           | 0.06              | 0         | 196                 |
| AB +               | 22  | Male           | 28.2        | 0                           | 17.48             | 5.5       | 160                 |
| AB +               | 23.8| Female         | 32.9        | 0                           | 3.19              | 5.2       | 287                 |
| T2D                | 36.1| Male           | 30.6        | 0                           | 3.45              | 7.2       | 332                 |
| T2D                | 42.8| Male           | 31          | 2                           | 0.58              | 7.8       | 400                 |
| T2D                | 45  | Female         | 32.3        | 15                          | 4.17              | 0         | 209                 |
| T2D                | 48  | Male           | 41          | 2                           | 3.46              | 0         | 247                 |
| T2D                | 45  | Female         | 39.1        | 2                           | 3.17              | 0         | 286                 |
| T2D                | 62  | Female         | 19.9        | 10                          | 6.14              | 6         | 265                 |

Continued
SNP analysis. Genotyping data were retrieved from the UCSD T1D GWAS meta-analysis which includes samples from 501,638 control individuals and 18,942 patients with T1D. Similarly, the T2D multi-ethnic meta-analysis includes samples from nearly 1.2 million control subjects and 228,499 T2D cases.

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.

Ethical 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).

Data availability. Data have been deposited with datadryad.org https://doi.org/10.5061/dryad.d7wm37q4h. 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.

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Author contributions
K.B. conceptualized the project and together with H.T. and K.J. wrote the original manuscript draft. L.K., K.D.J., and I.G. provided the analyzed material and performed the RNA expression analysis. F.P. performed the SNP analysis. All authors edited, reviewed, and approved the final manuscript.

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Competing interests
The authors declare no competing interests.

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