Journal of Autoimmunity 156 (2025) 103468

Contents lists available at ScienceDirect

Journal of Autoimmunity

journal homepage: www.elsevier.com/locate/jautimm

Epigenetic differences at immune and type 1 diabetes susceptibility genes in blood from young children after COVID-19 infection

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ARTICLE INFO

Handling editor: C Selmi

Keywords:
Type 1 diabetes
Autoimmunity
Viral infection
COVID-19
SARS-CoV-2
Influenza
DNA methylation

ABSTRACT

Background: Viral infections, including COVID-19, are associated with an increased risk for type 1 diabetes (T1D), but potential underlying mechanisms remain unexplored. We evaluated whether COVID-19 or influenza A infection is characterized by differential DNA methylation at immune and T1D susceptibility genes in young children at risk for T1D.

Methods: Epigenome-wide association analysis using the Illumina MethylationEPIC microarray was performed in blood taken at age 1.5 years (IQR, 1.49–1.52 y) from 740 prospectively followed children with increased risk of T1D. SARS-CoV-2 and influenza A H1N1 antibodies were monitored at 2–4-month intervals from age 6 months to identify infection.

Results: COVID-19 and influenza infection occurred prior to the DNA methylation sample in 81 and 74 children, respectively. Of these, infection occurred within 3 months of the DNA methylation sample (recent infection) in 43 and 22 children. Compared to children without COVID-19 or influenza A infection, children with recent COVID-19 infection showed differential methylation at key immune- and antiviral genes, including *ADAR*, *IFI44L*, *MX1* and *OASL*. In addition to *ADAR*, six further T1D susceptibility genes, including the SARS-CoV-2 cell entry receptor neuropilin-1, had differential methylation at nearby CpGs in children infected by SARS-CoV-2. A quantitatively less differential methylation was also observed in children with an earlier COVID-19 infection at some of these CpG sites. Infections with influenza showed no associations.

Conclusion: Children with SARS-CoV-2 infection showed sustained DNA methylation changes at genes critical for antiviral response and T1D susceptibility, potentially contributing to immune dysregulation and promotion of the autoimmune process underlying T1D.

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https://doi.org/10.1016/j.jaut.2025.103468
Received 30 May 2025; Received in revised form 17 July 2025; Accepted 26 July 2025
Available online 31 July 2025

1. Introduction

Type 1 diabetes (T1D) is one of the most common autoimmune diseases in children and adolescents, characterized by immune-mediated destruction of pancreatic islet beta cells. The underlying autoimmune process is initiated by gene-environment interactions [1]. There is a strong polygenic background associated with the development of T1D, notably at immune-related and antiviral gene loci [2]. However, the specific environmental factors that contribute substantially to the development of T1D have yet to be clearly identified [3].

Viral infections may cause and promote the autoimmune process [4]. Several virus species have been linked to the development of T1D, including coxsackievirus B [5], influenza A [6], and SARS-CoV-2 [7]. The COVID-19 pandemic was paralleled by an increase in T1D incidence across multiple countries [8–11]. More specifically, COVID-19 infections in early childhood were associated with the risk of developing islet autoimmunity [12] and accelerated the autoimmune process in children with a presymptomatic stage of T1D [13]. Like other virus types, it has been shown that SARS-CoV-2 is able to infect human pancreatic beta cells [14], potentially initiating immune response mechanisms but also promoting autoimmune processes, for example by epitope spreading or bystander activation [4].

The immune response to viral infections needs tight regulation to efficiently eliminate the infectant without damaging healthy cells. Expression of immune-related genes is controlled by multiple regulatory layers, including transcription factor activity and epigenetic mechanisms, such as DNA methylation [15]. It therefore is essential that the regulatory machinery returns to the pre-infection status to prevent the possibility of chronic immune activation. With respect to COVID-19 infection, it has been shown that severely affected patients show altered blood DNA methylation at genes known to be important for the immune response [16–18]. Moreover, differential blood DNA methylation at antiviral genes has been observed months after the infection in adults [17,18]. A prolonged pro-inflammatory gene expression signature has also been discovered in single immune cell types of COVID-19 patients [19]. This suggests that viral infections can induce acute and lasting epigenetic changes with potential side effects for the host, such as altered regulation of genes that have been implicated in autoimmune diseases.

In this study, we evaluated whether a previous COVID-19 or influenza A infection associated with epigenome-wide blood methylation differences, especially at immune and T1D susceptibility genes, in young children with an elevated risk of T1D.

2. Methods

2.1. POInT study

POInT is a randomised, controlled multicentre clinical trial organised through the Global Platform for the Prevention of Autoimmune Diabetes (GPPAD) [20]. It is investigating whether daily intake of oral insulin reduces the incidence of islet autoimmunity and/or T1D in children with an increased genetic risk of T1D (ClinicalTrials.gov registration no. NCT03364868). Between February 2018 and March 2021, a total of 1050 infants were enrolled at 4.0–7.0 months of age. Follow-up visits were performed at 2, 4 and 8 months after the baseline visit, at 1.5 years of age and then every 6 months until the maximum age of 7.5 years. A whole blood DNA sample for methylation assessment was collected at the 1.5-year visit in 794 children for whom consent was given to use their DNA for genetic research and whose DNA was of adequate quality and quantity (Supplementary Fig. S1). Based on the time of enrollment, the last child included in this analysis reached the 1.5-year visit in May 2023. None of the children received vaccination against SARS-CoV-2 before the 1.5-year visit.

POInT was approved by the institutional review boards and regulatory authorities of the Technische Universität München, Medical Faculty (326/17 Af), the Medical University of Warsaw (199/2017), the UK Health Research Authority (18/SC/0019), Onderzoek UZ/KU Leuven (S60711), and Regionala Etikprövningsnämnden i Lund (2017/918). Written informed consent to participate in the study was obtained from all the study participants legal representatives. A separate informed consent from the participating families was required to allow biobank storage of material, such as the DNA used in this study.

2.2. Viral antibody measurements

Antibodies against SARS-CoV-2 and influenza A (H1N1) were measured using luciferase immunoprecipitation system (LIPS) assays as described previously [12]. IgG SARS-CoV-2 antibodies to the receptor binding domain (amino acids 309–544 of the original variant sequence), the S2 portion of the spike protein (amino acids 697–1224), and the nucleocapsid protein (amino acids 1–422) were measured at the Dresden University of Technology, Germany. Serum samples at the 2-year visit of 885 children were screened for antibodies against the receptor binding domain protein. Positive samples, i.e., $\geq$1 arbitrary unit (equivalent to 5 binding antibody units), were subsequently tested against the S2 and the nucleocapsid protein. Samples were defined as positive for SARS-CoV-2 antibodies if they were positive against the receptor binding domain and the nucleocapsid protein antigens. Antibodies to the influenza A (H1N1) virus (A Germany 7405 2018) HA1 antigen (amino acids 19–344 of HA) were measured in a similar format. If children tested positive for SARS-CoV-2 or influenza A (H1N1) antibodies at the 2-year visit, samples collected at earlier visits were tested to determine the earliest positive test result.

2.3. DNA methylation measurements

Genomic DNA was extracted from whole blood and 750 ng bisulfite-converted using the EZ 96-DNA methylation kit (Zymo Research, La Jolla, CA, USA). Genome-wide DNA methylation analysis using the Infinium MethylationEPIC (850K) Bead-Chip array (Illumina, San Diego, CA, USA) was performed at Life & Brain GmbH (Bonn, Germany). The Bead-Chips were imaged using an Illumina iScan and the raw fluorescence intensities of the images were extracted using the GenomeStudio Methylation module (Illumina).

Pre-processing and quality control were performed using R software (version 4.3.2) with the R package *ENmix*. The methylation probes were excluded from downstream analysis if their detection *P*-value was $>10^{-6}$ and if the bead count was less than three in at least 5 % of the sample. Data were normalized using quantile normalization as implemented in *ENmix*. We excluded probes on sex chromosomes, cross-hybridizing probes, probes located near single nucleotide polymorphisms [21] and probes with more than 20 % missing data. The final set included 689,919 cytosine-phosphate-guanine (CpG) dinucleotides.

2.4. Epigenome-wide association analysis

The associations between the children's blood DNA methylation and viral infection status (non-infected group coded as "0" and infected group as "1") were analyzed by robust linear regression using the R package *limma*. Methylation M-values (logit-transformed β values) were used for all analyses. Principal component analysis using all samples and included probes was performed and the first three components were used to account for potential technical variation. Frequencies of six blood cell types ($CD4^+$ T cells, $CD8^+$ T cells, B cells, natural killer cells, monocytes and neutrophils) were estimated from a reference panel using the R package *FlowSorted.Blood.EPIC*. Regression models were adjusted for age at DNA sample, sex, study site, the first three principal components, and the six estimated blood cell types. Extreme methylation outliers were excluded based on the Tukey method. The false discovery rate (FDR) according to Benjamini-Hochberg was used to account for multiple testing. Statistical significance was set at FDR <0.05.

2.5. Functional evaluation of infection-associated CpGs

CpG-annotated genes were checked for their immune-related function using the genes included in the gene ontology "immune system process" (GO:0002376). Gene ontology and Reactome Pathway Database analysis were performed using Panther [22]. Enrichment of ontologies or pathways among the input genes or proteins was assessed using Fisher's exact test and significance set at FDR <0.05. Publicly available blood *cis*-expression quantitative methylation (eQTM) data (Illumina 450K array; blood cell type adjusted) from 832 children (mean age 8.1 years) participating in the Human Early Life Exposome (HELIX) project were used to investigate CpG target genes and associations between methylation and gene expression levels. The public epigenome-wide association study (EWAS) catalog [23] was queried for previous associations between the infection-associated CpGs and protein and phenotypic traits. Trait enrichment analysis among the infection-associated CpGs was performed by hypergeometric tests using the *phyper* function in R software. Correction for multiple testing was done using the Bonferroni method ($P < 0.05/252$ traits).

2.6. T1D susceptibility gene analysis

For the targeted analysis of CpGs at T1D susceptibility genes, 431 genes associated with T1D were retrieved from the Harmonizome database [24] and the NHGRI-EBI GWAS catalog. The 13,788 CpGs annotated to these 431 genes were selected for a differential methylation analysis as described above. The FDR was used to account for multiple testing.

3. Results

3.1. Infection status

Among the 740 children with available DNA methylation and antibody data, 81 had developed antibodies to SARS-CoV-2 consistent with COVID-19 infection by the time of sampling for DNA methylation. These included 43 who developed SARS-CoV-2 antibodies at (recent COVID-19 infection group) and 38 prior to (earlier COVID-19 infection group) the visit when the DNA methylation was assessed (Table 1; Supplementary Fig. S1). Antibodies to H1N1 HA1 consistent with influenza A infection were observed in 74 children at (n = 22, recent influenza infection group) or prior to (n = 52, earlier influenza infection group) the DNA methylation sample (Table 1; Supplementary Fig. S1). Two additional children had both infections and were excluded from the analysis.

3.2. COVID-19 infection associates with differential methylation at immune-related genes

In comparison to children without COVID-19 or influenza A infection, the 81 children with previous COVID-19 exhibited 98 differentially methylated CpGs (FDR <0.05). These included 16 with annotation to immune-related genes such as *IFI44L*, *IFITM1* and *MX1* (Supplementary Table S1; Supplementary Fig. S2A). There was no enriched immune-related gene ontology among the annotated genes. The CpG gene targets *MX1* and *MX2* were examined in the eQTM data where the observed hypomethylation in children with COVID-19 at these genes was associated with higher expression of *MX1* and *MX2* (Supplementary Table S1).

To assess whether changes were likely to be sustained, DNA methylation was examined in children stratified by whether the infection was recent or earlier. In the recent COVID-19 infection group, differential DNA methylation was observed at 45 CpGs (Supplementary Table S2; Fig. 1A). Twenty of the 45 CpGs are annotated to immune-related genes, including the antiviral genes *ADAR*, *IFI44L*, *IFITM1*, *IFIT3*, *MX1* and *OASL* (Supplementary Table S2). The CpG target genes *IFI44*, *IFI44L*, *MX1* and *MX2* were examined in the eQTM data. Hypomethylation at these genes was observed in children with recent COVID-19 infection which was associated with higher gene expression in the eQTM data (Supplementary Table S2). Gene ontology analysis using all 37 annotated genes showed enrichment of interleukin-27-mediated signaling, regulation of viral genome replication, viral processes and life cycle as well as defense against viral infections (FDR <0.05; Fig. 1B–Supplementary Table S3). Furthermore, enrichment analysis among protein traits associated with the 45 CpGs resulted in 45 enriched traits, including 23 immune-related proteins (hypergeometric test, $P_{Bonferroni} < 0.05$; Supplementary Table S4). Reactome analysis of these 45 proteins showed enrichment in interferon-alpha, -beta and -gamma signaling, antiviral mechanisms, and SARS-CoV-2 infection pathways (FDR <0.05; Fig. 1C–Supplementary Table S5). Exploration of the EWAS catalog for non-protein traits additionally revealed enrichments for seven phenotypic traits, four of which were autoimmune diseases, namely systemic lupus erythematosus, multiple sclerosis, rheumatoid arthritis and primary Sjogren's syndrome (Supplementary Table S4).

The 45 CpGs differentially methylated in recently infected children were examined in the 38 children with earlier COVID-19 infection to assess whether there was a prolonged association between infection and DNA methylation beyond the period of infection. The effect estimates in the recent and earlier infection groups were correlated for these 45 CpG sites and differed significantly also in the earlier infection group for five sites (Pearson's r = 0.56, $P < 0.001$; Supplementary Fig. S3). For all the 45 CpG sites, the effect estimates were lower in the earlier infection group than in the recent infection group (Mann-Whitney-U test, $P < 0.001$).

To examine whether the methylation profiles were present before COVID-19 infection and may have contributed to infection susceptibility, we analyzed the methylation status of 98 and 45 CpG sites associated with previous or recent infection in 61 children who had their first COVID-19 infection in the 6 months after the DNA methylation sample. No significant methylation differences at these CpG sites were observed prior to infection when compared to the remaining 522 children who had no COVID-19 infection (Supplementary Table S6). Children infected by influenza A, irrespective of the time post-infection, showed no methylation differences compared with the non-infected children (Fig. 1D; Supplementary Fig. S2B). To assess similarities between

Table 1
Characteristics of the study sample.

	Children without COVID-19 or influenza A infection	All children with COVID-19 infection	Children with recent COVID-19 infection	Children with earlier COVID-19 infection	All children with influenza A infection	Children with recent influenza A infection	Children with earlier influenza A infection
Total n	583	81	43	38	74	22	52
Age at DNA sample (y)	1.50 (1.48–1.51)	1.51 (1.49–1.52)	1.51 (1.49–1.52)	1.50 (1.50–1.50)	1.50 (1.49–1.52)	1.50 (1.49–1.51)	1.50 (1.50–1.63)
Female – n (%)	290 (49.7)	40 (49.4)	21 (48.8)	19 (50.0)	44 (59.5)	14 (63.6)	30 (57.7)
Age at viral antibodies (y)	n/a	1.48 (0.88–1.51)	1.51 (1.49–1.53)	0.87 (0.70–1.13)	1.10 (0.72–1.48)	1.50 (1.49–1.51)	0.85 (0.65–1.15)

Data is presented as median (IQR) or n (%).

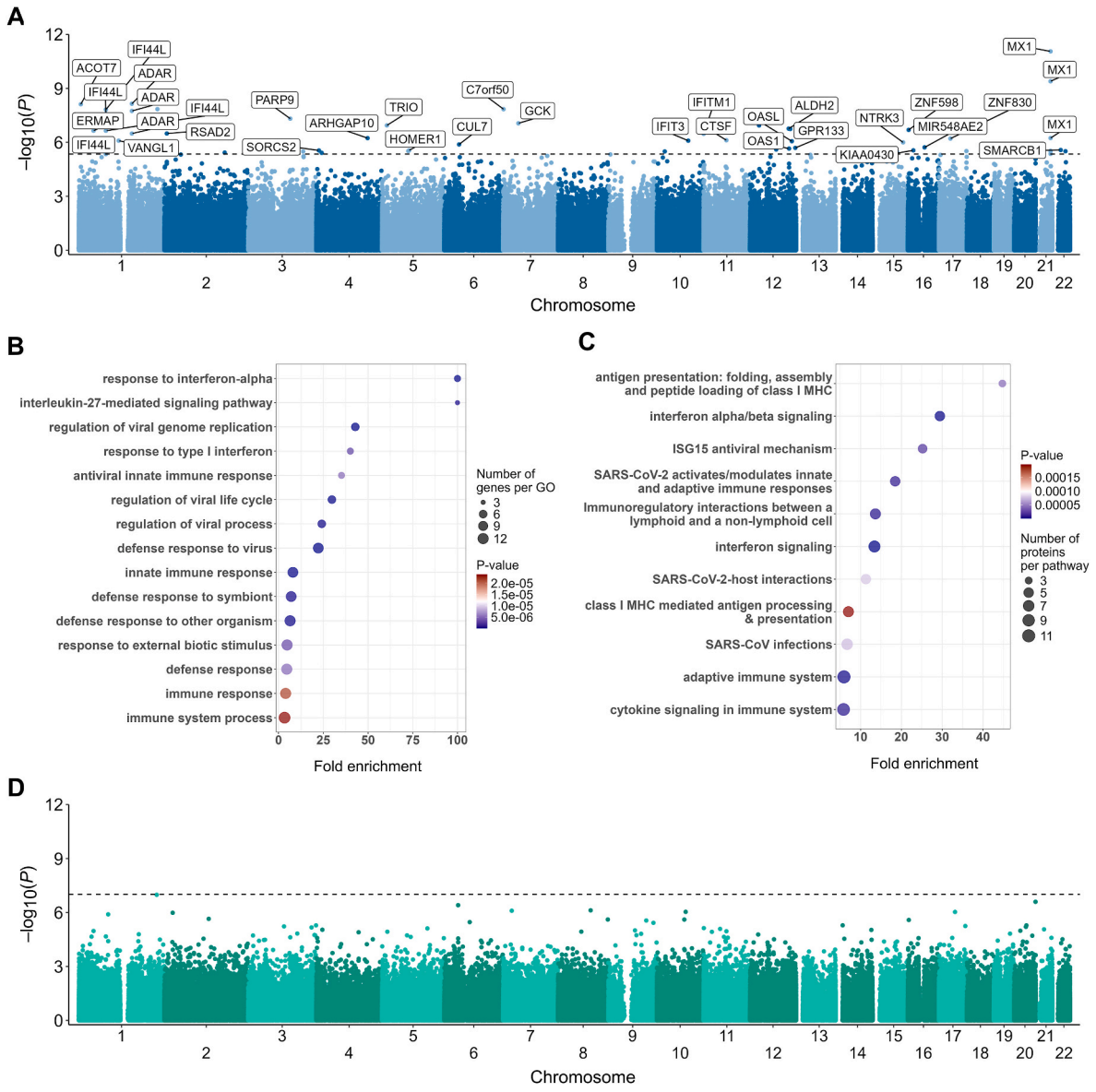

Fig. 1. Epigenome-wide associations with COVID-19 and influenza A infections in young children. **A**, Manhattan plot showing the $-\log_{10}(P)$ of the associations between the children's blood DNA methylation status of individual CpG sites across the autosomal chromosomes (1–22) and a recent COVID-19 infection (within 3 months prior to the DNA sample; n = 43 vs. 583 children without infection). **B**, Enriched gene ontologies (GO) among genes targeted by differential methylated CpGs in children with a recent COVID-19 infection (within 3 months prior to the DNA sample; n = 43 vs. 583 children without infection). **C**, Enriched Reactome Database Pathways among proteins associated with differential methylated CpGs in children with a recent COVID-19 infection (within 3 months prior to the DNA sample; n = 43 vs. 583 children without infection). **D**, Manhattan plot showing the $-\log_{10}(P)$ of the associations between the children's blood DNA methylation status of individual CpG sites across the autosomal chromosomes (1–22) and a recent influenza A infection (within 3 months prior to the DNA sample; n = 22 vs. 583 children without infection). Horizontal dotted line indicates the FDR threshold. Top CpG annotations are shown.

influenza A and COVID-19 infections, the 100 CpGs with lowest nominal *P*-value were checked for overlapping CpGs. Only one CpG (cg05696877) at *IFI44L* was commonly associated with both infections and showed the same effect direction in both settings (previous influenza infection: estimate = −0.56, SE = 0.13, *P*-value = 2.1 x 10^{-05} and recent COVID-19 infection: estimate = −0.70, SE = 0.15, *P*-value = 4.5 x 10^{-06}).

3.3. *COVID-19 infections associate with CpG methylation at T1D susceptibility genes*

In children with previous COVID-19 infections, differential methylation was identified at six CpGs linked to six T1D susceptibility genes, *AGPAT1*, *ATF6B*, *CNTNAP2*, *FCRL3*, *NRP1* and *RAD51B* (FDR <0.05; Fig. 2A). The associations with the six CpGs were nominally significant ($P < 0.05$) in both stratified subsets of recent and earlier COVID-19 infection, and effect estimates correlated strongly between the recent and earlier infection groups (Pearson's $r = 0.98$, *P*-value <0.001; Fig. 2B). No differential methylation at T1D susceptibility gene loci was detected in children with previous influenza A infections (Supplementary Fig. S4).

4. Discussion

Viral infections can induce lasting health consequences for the host and have been associated with the development of multiple autoimmune diseases, including T1D. Differential DNA methylation has been observed in adults after a COVID-19 infection [18,25]. In this analysis,

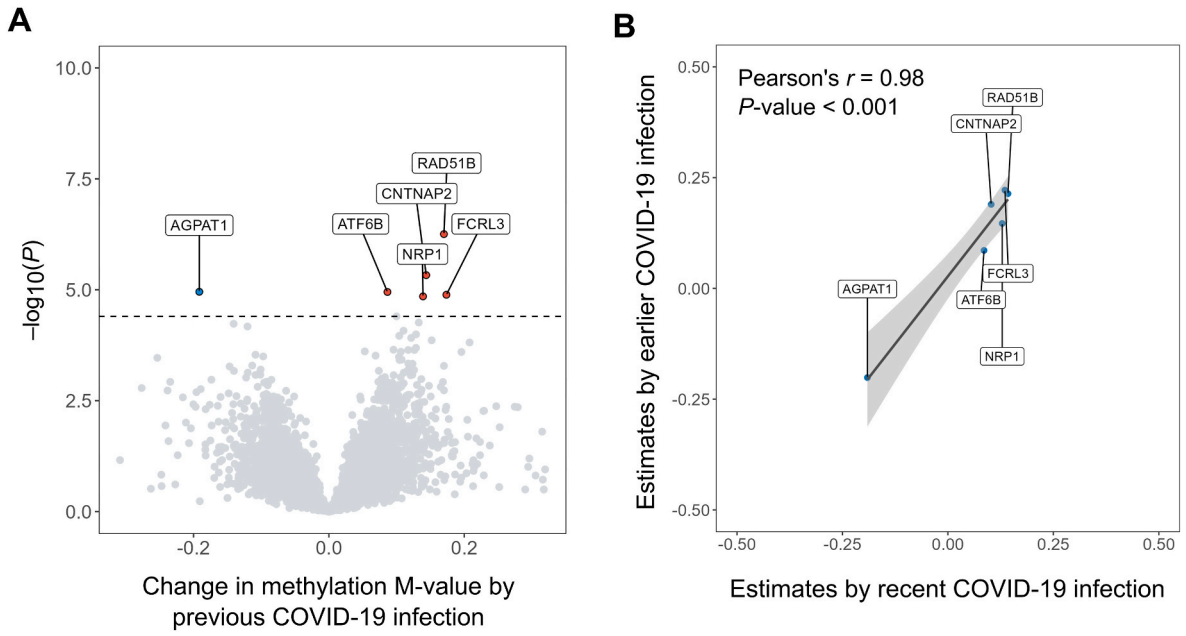

Fig. 2. Differential methylation at type 1 diabetes susceptibility genes in children following COVID-19 infection. A, Volcano plot showing $-\log_{10}(P)$ against the effect estimates (change in methylation M-value by previous COVID-19 infection; n = 81 vs. 583 children without infection) of 13,788 CpGs at 431 T1D susceptibility genes derived from the Harmonizome database and the NHGRI-EBI GWAS catalog. Horizontal dotted line indicates the FDR threshold. Top CpG annotations are shown. **B**, Correlation plot of effect estimates of six CpGs at T1D susceptibility genes with differential methylation in previously COVID-19 infected children (any time prior to DNA sample; n = 81 vs. 583 children without infection) between children with recent (within 3 months prior to DNA sample; n = 43 vs. 583 children without infection) and earlier (over 3 months prior to DNA sample; n = 38 vs. 583 children without infection) COVID-19 infection.

we showed that in very young children, aged 5–30 months, COVID-19 infections were associated with methylation changes, especially at relevant immune and antiviral genes. This was particularly evident in children with recent infections, but changes persisted for up to 12 months after a COVID-19 infection. We also observed differential methylation at T1D susceptibility genes in these children. In contrast, no associations with the epigenetic loci were seen for influenza A infections.

COVID-19 was associated with methylation changes at several immune genes relevant to antiviral defense, as highlighted by the enriched gene ontologies and Reactome Database Pathways. Epigenetic alterations at various antiviral genes, such as *IFI44L* and *MX1*, have been described previously in adult patients with severe COVID-19 infections [25]. It is notable that these effects were similarly seen in young children who generally experience mild COVID-19 infections [26]. We observed a strong enrichment of interleukin-27-mediated signaling, potentially indicating higher circulating IL-27 levels in recently infected children. Indeed, elevated IL-27 levels have been found in COVID-19 patients [27]. Balanced regulation of this cytokine is critical to promote cytotoxic events against the infectious agent but also to prevent unnecessary tissue damage [27]. IL-27 plays a key role in controlling $Foxp3^+$ regulatory T (Treg) cell functions in chronic inflammation and autoimmunity. Mice lacking the IL-27 receptor alpha subunit ($Il27ra^{-/-}$) in Tregs, spontaneously developed autoimmune encephalitis and systemic administration of IL-27 is unable to ameliorate chronic inflammation in these animals [28]. Also, mice infected by pathogens, such as *Toxoplasma gondii*, depended on functional IL-27-induced Tregs to limit effector T cell mediated inflammation [29]. The induction of peripheral immune tolerance by Tregs is critical in such conditions and may be affected following COVID-19 infection [30]. Depleted levels of Tregs have been observed in patients with COVID-19 [31]. Dysregulation of IL-27 (higher and lower levels) has been associated with the development of several autoimmune diseases [32] and may promote T1D development via alterations of Treg functions [30].

In our analysis, most immune-related changes were more pronounced in recently infected children, indicating that the epigenetic machinery enabling the antiviral response possibly returns to pre-infection status after months. However, CpGs at antiviral genes, such as *IFI44L*, showed methylation differences when investigating children with earlier COVID-19 infections. This is in line with previous studies on longitudinal data in adults that showed persistent methylation changes for several months, for example at *IFI44L* [17,18]. Together with findings on persistent transcriptomic alterations [19], this suggests that SARS-CoV-2 infections induce lasting modifications of the immune gene regulation, potentially enabling fast reaction to reinfections. This permanent active state, however, may also promote hypersensitivity of the immune system and increased susceptibility to autoimmune disease.

There are many possibilities through which immune perturbations may contribute to the etiology of T1D. A recent study highlighted the role of RNA-editing enzymes in the interplay between viral infections and the onset of T1D [33]. One of the major RNA-editing and antiviral enzymes is ADAR, which promotes intracellular viral double-stranded RNA disruption and suppresses the infection-triggered interferon response, balancing the viral-induced immune reaction [34]. In mice, pancreatic beta-cell specific knock-out of *Adar* mimicked features of an early-stage T1D, such as massive interferon production followed by islet inflammation and beta cell loss [33]. This suggests that dysfunction of ADAR represents a potential mechanism in the development of T1D. In the children with recent COVID-19 infection, we found three CpGs with differential methylation at *ADAR* in blood cells, illustrating that epigenetic regulation at this gene may be affected by SARS-CoV-2, potentially also in beta cells. In our targeted approach we also found alterations at known T1D susceptibility genes in children with COVID-19 infections. Among the affected genes, *neuropilin-1* (*NRP1*) is of particular interest, as this receptor facilitates the cell entry of SARS-CoV-2 [35]. Neuropilin-1 is highly expressed and a specific surface marker in $CD4^+CD25^+$ Tregs [36] and is vital for Treg stability and their immunosuppressive function [37]. Furthermore, human pancreatic beta cells highly express *NRP1* and pharmacological blocking of this receptor prevented SARS-CoV-2-induced beta cell dysfunction and loss [14]. The human Fc receptor-like 3 (*FCRL3*) gene is a known T1D susceptibility locus and has been implicated in the risk for multiple autoimmune

diseases [38]. Stimulation of FCRL3 in Tregs has been shown to impair their suppressive function on effector T cell proliferation, promoting Treg dysfunction and loss of immune tolerance [39,40]. Together with other COVID-19-associated gene targets identified in this study, these findings point to a potential impact on Treg biology, potentially providing a mechanistic link between COVID-19 infection and increased T1D risk.

The collection of epigenetic samples at the 1.5-year visit restricted us to events occurring before May 2023. In the first years of the COVID-19 pandemic in Europe, children were less frequently infected than adults, and the spread of other viruses, including influenza, was limited due to pandemic-related restrictions. Moreover, the cross-sectional design of this analysis precludes conclusions about longer persistence of epigenetic alterations or whether the methylation differences were already apparent prior to the infection. The analysis in 61 children with pre-infection blood samples indicated no methylation differences prior to COVID-19 infection. In addition, the overlap of several key antiviral genes associated with COVID-19 infections with those identified in previous longitudinal studies suggests that some epigenetic changes may be stable over time. Gene expression data were unavailable for our participants, restricting our ability to confirm CpG–gene relationships and draw more definitive conclusions. Therefore, we used publicly available blood eQTM data to validate gene targets of COVID-19-associated CpGs. However, the eQTM data was based on the Illumina 450K array limiting our ability to evaluate many CpGs, as most were only present on the EPIC array. Access to primary T1D-relevant cells or tissues, including specific immune cell subsets or pancreatic islets, was not feasible in this study, limiting our observations to whole blood cells. This analysis was performed in children with a higher genetic risk for T1D and the observations may differ in children with lower susceptibility. Last, due to the associative nature of this analysis, we cannot draw conclusions about causality.

To conclude, we identified in children with a high risk for T1D substantial associations between blood epigenetic modifications at relevant immune and antiviral genes and previous COVID-19 infection, but not after influenza A infection. Resultant immune perturbations and contributions to the development of T1D remain to be investigated.

CRediT authorship contribution statement

Raffael Ott: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Conceptualization. **Tarini Singh:** Methodology, Formal analysis. **Katharina Schütte-Borkovec:** Writing – review & editing, Resources, Investigation. **Marlon Scholz:** Resources, Project administration. **Kristina Casteels:** Writing – review & editing, Resources, Investigation. **Gita Gemulla:** Writing – review & editing, Resources, Investigation. **Olga Kordonouri:** Writing – review & editing, Resources, Investigation. **Helena Elding Larsson:** Writing – review & editing, Resources, Investigation. **Agnieszka Szypowska:** Writing – review & editing, Resources, Investigation. **John A. Todd:** Writing – review & editing, Resources, Investigation. **Anette-G. Ziegler:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization. **Ezio Bonifacio:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization.

Funding

This work was funded by the EASD-Novo Nordisk Foundation Diabetes Prize for Excellence NNF22SA0081044 to AGZ (Helmholtz Munich). The POInT clinical trial was funded by The Leona M. and Harry B. Helmsley Charitable Trust (Helmsley) grants 2018 PG-T1D022 (GPPAD-02 study and GPPAD coordinating center), 2018 PG-T1D023 (GPPAD-03 study: POInT – Primary Oral Insulin Trial), 2018 PG-T1D062 (Biobanking for POInT) and 2003–04286 (GPPAD coordinating center continuation). The funders had no role in the design, data collection, data analysis, and reporting of this study.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Anette-G. Ziegler reports a relationship with Novo Nordisk that includes: consulting or advisory. Anette-G. Ziegler reports a relationship with Sanofi-Aventis that includes: consulting or advisory and speaking and lecture fees. Ezio Bonifacio reports a relationship with Sanofi-Aventis that includes: speaking and lecture fees and travel reimbursement. Helena Elding Larsson reports a relationship with Novo Nordisk that includes: speaking and lecture fees. Helena Elding Larsson reports a relationship with Sanofi-Aventis that includes: speaking and lecture fees. Gita Gemulla reports a relationship with Sanofi-Aventis that includes: speaking and lecture fees. Olga Kordonouri reports a relationship with Amgen that includes: consulting or advisory and speaking and lecture fees. Olga Kordonouri reports a relationship with Dexcom that includes: consulting or advisory and speaking and lecture fees. Olga Kordonouri reports a relationship with Diamyd Medical that includes: consulting or advisory and speaking and lecture fees. Olga Kordonouri reports a relationship with Sanofi-Aventis that includes: consulting or advisory and speaking and lecture fees. Agnieszka Szypowska reports a relationship with Synoptis Pharma that includes: speaking and lecture fees and travel reimbursement. Agnieszka Szypowska reports a relationship with Sanofi-Aventis that includes: consulting or advisory and speaking and lecture fees. Agnieszka Szypowska reports a relationship with Proglikemia that includes: consulting or advisory. Agnieszka Szypowska reports a relationship with Medtronic Poland that includes: consulting or advisory. Anette-G. Ziegler is listed as inventor for the patent "Method for determining the risk to develop type 1 diabetes" (WO 2019/002364) by Helmholtz Zentrum München Deutsches Forschungszentrum für Gesundheit und Umwelt (GmbH) and Technical University of Dresden. Ezio Bonifacio is listed as inventor for the patent "Method for determining the risk to develop type 1 diabetes" (WO 2019/002364) by Helmholtz Zentrum München Deutsches Forschungszentrum für Gesundheit und Umwelt (GmbH) and Technical University of Dresden. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We sincerely thank the families participating in the POInT study for supporting T1D research. We also thank the primary care pediatricians and physicians for supporting the study. We are grateful to all study teams for their enduring efforts. POInT Study group: GPPAD-Coordinating Centre (CC): Peter Achenbach, Stefanie Arnolds, Karina Blasius, Ezio Bonifacio, Michaela Dilong, Veronika Fendt, Melanie Gündert, Florian Haupt, Martin Heigermoser, Maja Hergl, Elisabeth Huber, Sandra Hummel, Nadine Klein, Ramona Lickert, Claudia Matzke, Lorena Müller, Rebecca Niewöhner, Marlon Scholz, Katharina Schütte-Borkovec, Merve Vurucu, José Maria Zapardiel Gonzalo, Anette-Gabriele Ziegler. Statistical analysis team: Thomas Lang, Markus Pfirrmann (independent statistician, DSMB), Andreas Weiβ (trial statistician). Screening for genetic T1D risk (GPPAD-02): Belgium, Flanders: Kristina Casteels, An Jacobs, Charlien Jannsen, Hilde Morobé, Jasmin Paulus, Anne Rochtus, Natalie Van den Driessche, Renka Van Heyste, Veerle Vanhuyse, Brontë Vrancken; Germany, Saxony: Uta Ceglarec, Franziska Ehrlich, Angela Hommel, Anja Loff, Peter Mirtschink, Pauline Wimberger, Nicole Zubizarreta; Germany, Lower Saxony: Nils, Janzen, Frank Roloff; Germany, Bavaria: Annika Fehn, Stefanie Jacobson, Elisabeth Huber, Christiane Winkler; Poland, Voivodship Mazowieckie: Marta Krysicka, Mariusz Ołtarzewski, Agnieszka Szypowska; Sweden, Skåne Region: Helena Elding Larsson, Ida Jönsson, Dorota Lacki, Markus

Lundgren, Hannah Nenonen; UK, Oxford Region: Parvinder Aley, Ian Smith, Matthew Snape, Nazia Taj, John A Todd, Manu Vatish, Tabitha Wishlade. POInT study (GPPAD-03): Belgium, Leuven: Kristina Casteels, Janne Houben, An Jacobs, Charlien Jannsen, Jasmin Paulus, Anne Rochtus, Natalie Van den Driessche, Veerle Vanhuyse, Brontë Vrancken; Germany, Dresden: Sari Arabi, Reinhard Berner, Ruth Blechschmidt, Ezio Bonifacio, Sevina Dietz, Franziska Ehrlich, Anne Eugster, Gita Gemulla, Sophie Heinke, Raphael Hoffmann, Angela Hommel, Susan Kowal, Franziska Lange, Anja Loff, Marija Lugar, Marie-Luise Michael, Bianca Schlee, Marc Weigelt, Nicole Zubizarreta; Germany, Hanover: Laura Galuschka, Olga Kordonouri, Carolin Kruse, Sarah Landsberg, Erika Marquardt, Mareike Niemeyer, Felix Reschke, Frank Roloff, Bianca Schmidt, Kerstin Semler, Thekla von dem Berge, Jantje Weiskorn; Germany, Munich: Peter Achenbach, Melanie Bunk, Willi Grätz, Anna Hofelich, Annette Knopff, Annette Munzinger, Claudia Ramminger, Franziska Reinmüller, Cigdem Sanverdi, Katharina Sarcletti, Sarah Schill, Anette-Gabriele Ziegler; Poland, Warsaw: Adrianna Cieloch, Wiktoria Czerwińska, Sylwia Dybkowska, Natalia Dziedzic, Elżbieta Górska, Lidia Groele, Agnieszka Mroczek, Dorota Owczarek, Katarzyna Popko, Agnieszka Szypowska, Beata Zduńczyk, Anna Zych; Sweden, Malmö: Sofie Alström Mortin, Susanne Dahlberg, Helena Elding Larsson, Lina Fransson, Malin Goldman-Tsubarah, Markus Lundgren, Jessica Melin, Hannah Nenonen, Kobra Rhamati, Falastin Salami, Carina Törn; UK, Oxford: Parvinder Aley, Owen Bendor-Samuel, Rachel E J Besser, Rachel Craik, Sophia Hawkins, Alice Lelliott, Helen Ratcliffe, Matthew Snape, John A Todd, Sophie Vernon, Connor Whelan, Louise Willis.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jaut.2025.103468.

Data availability

We have included the relevant summary statistics within the main text and supplementary tables. Full summary statistics of the EWAS models are available at the EWAS catalog (Zenodo doi: https://doi.org/10.5281/zenodo.15494278). The publicly available data used in this study can be accessed from the EWAS catalog (https://www.ewascatalog.org/download/) and the HELIX project (https://helixomics.isglobal.org/).

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