ISPAD-Breakthrough 2024 Research Fellowship – Progress Report

Project title:

The interplay between glycemic variability and epigenetics in adolescents with type 1 diabetes

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Introduction

Early metabolic control in pediatric type 1 diabetes (T1D) shapes long-term outcomes through the phenomenon of metabolic memory. Epigenetic mechanisms, particularly DNA methylation, are strongly implicated as the molecular basis; yet, prospective evidence capturing the dynamic formation of these marks in the early years after diagnosis remains limited.

During the course of this fellowship, I:

(1) focused on advancing methodological standards for continuous glucose monitoring (CGM) data analysis through the further development of GlyCulator 3.0,
(2) conducted a comprehensive evaluation of public DNA methylation datasets from individuals with and without diabetes, and
(3) expanded my recruitment efforts within ongoing projects of epigenome-wide profiling in children with T1D.

Together, the undertaken actions aim to characterize the respective effects of hyperglycemia (as measured by HbA1c) and glycemic variability (as measured with CGM) on epigenetic remodeling and to explore the potential partial reversibility of such changes.

In parallel, I pursued epidemiological research, including a 40-year longitudinal analysis of incidence and prevalence trends at the central pediatric diabetes reference center in Poland, as well as a nationwide cross-sectional study assessing the prevalence of diabetic ketoacidosis (DKA) at the onset of type 1 diabetes (T1D). I have also continued my work on optimizing protocols for insulin pump use during physical activity in children with T1D. Altogether, these projects and actions provide a comprehensive approach to characterizing

and optimizing the management of T1D, highlighting critical areas that need improvement within the Polish clinical landscape.

Finally, I initiated a new research endeavor in the field of T1D pathogenesis and preventive strategies through a collaboration with the Leuven Diabetes Lab, focusing on the analysis of spatial transcriptomics datasets.

Below, I outline the main results and progress achieved in these projects during the ISPAD Breakthrough T1D fellowship, conducted at the Department of Biostatistics and Translational Medicine, Medical University of Lodz.

I. Standardization of CGM analysis and continued development of GlyCulator 3.0

As a lead developer and researcher within the GlyCulator 3.0 project, I have joined the international efforts of the CGM Metrics Consensus Group, led by Prof. Irina Gaynanova (University of Michigan, the "iglu" developer team). This collaboration focused on aligning definitions and analytical pipelines for CGM-derived metrics. I have refined a mathematical definition for CGM-based hypo- and hyperglycemia episodes, indicating that imprecision in the definition and implementation of those CGM metrics may result in clinically significant biases. The event-detection algorithm will be presented at the ISPAD 2025 Conference in Montreal (poster), with the respective manuscript to be submitted to Diabetes Technology & Therapeutics.

II. Metabolic Memory – recruitment, meta-analysis, and preliminary results

The prospective recruitment of children with T1D at the Pediatric Diabetes Reference Center in Lodz, Poland, is ongoing. The financial support from the Fellowship enabled the purchase of a blood sample collection method through TAP microneedle micro-sampling, which was introduced to minimize the burden among pediatric participants.

Concurrently, a meta-analysis of publicly available DNA methylation cohorts (8 studies, 2300 patients, ~51% with diabetes) was conducted. This analysis revealed novel CpG loci functionally associated with pathways related to inflammation, oxidative stress, and the DNA damage response, implicated in the development of diabetes complications. Those results will be presented at the ISPAD 2025 Conference in Montreal, Canada (poster).

The initial analysis of paired prospective samples (8 patients, two time points) demonstrated a dose–response relationship between HbA1c/CGM variability and methylation changes at selected CpG loci. Importantly, diverging methylation patterns were observed in patients with either deterioration or improvement in metabolic control within the 12-month follow-up period. These preliminary results suggest the potential for partial reversibility of epigenetic modifications and form the basis of a manuscript currently under preparation.

III. Other concurrent research efforts – epidemiology and physical activity optimization

Long-term incidence and prevalence of T1D in Central Poland

In collaboration with colleagues at the Department of Pediatrics in Lodz, I co-authored a 40-year prospective surveillance study (1983–2022) in which we systematically recorded all new-onset T1D cases in children under 15 in the Lodz voivodeship. This described the longest continuous pediatric diabetes registry in Poland. We demonstrated an over 10-fold increase in incidence, from 3.29/100,000 in 1983 to 32.4/100,000 in 2022, with two distinct periods of growth: a rapid rise between 1983 and 2005, followed by a slower but sustained increase thereafter. The burden has shifted toward older children (10–14 years), who now account for the majority of new cases. Seasonal variation was also evident, with diagnoses peaking in winter months. Prevalence reached 177/100,000 by 2022. These results were published in Hormone Research in Paediatrics (April 2025) [1].

National study of diabetic ketoacidosis (DKA) at T1D onset

To expand on the previous results, I also contributed to a nationwide multicenter study involving all 17 pediatric diabetes reference centers in Poland (2019–2022), capturing 6,543 cases of new-onset T1D. We found that over half of children (54.5%) presented in DKA, a rate higher than the European average. Using joinpoint regression and Bayesian forecasting, we showed that the COVID-19 pandemic exacerbated this trend – the observed DKA rates during 2020–2021 were 9.4 percentage points higher than predicted, and the severity of DKA was significantly higher, reflecting potential delayed diagnoses due to reduced healthcare access. Alarmingly, the incidence of DKA did not return to baseline after restrictions were lifted, stabilizing at ~54% in 2022. These findings underscore the pressing need for awareness campaigns, screening programs, and healthcare system enhancements to mitigate diagnostic delays in Poland. The manuscript is under submission, but the results will be presented (oral) at the European Academy of Pediatrics congress (Warsaw, Poland).

Physical activity and optimization of insulin delivery

Beyond epidemiology, I have conducted clinical intervention studies to optimize the use of diabetes technology during exercise in youth. In an initial study of adolescent football players, we developed and validated a novel protocol for hybrid closed-loop (HCL) use in contact sports, which combines temporary glucose targets with disconnection during training. The approach maintained good glycemic control (TIR ~79%) while improving time in the tight range (70–140 mg/dL) and reducing reliance on autocorrection boluses. These findings were published in Hormone Research in Paediatrics (October 2024) [2].

We have expanded on those initial results with a randomized pilot trial of children during a summer sports camp, using the HCL in two strategies: pump kept connected vs. temporary

disconnection during exercise. The results demonstrated superiority for pump disconnection in reducing hypoglycemia (TBR < 70 mg/dL, with a 1% margin) without compromising overall glycemic control (mean TIR ~85% in both groups). The results were presented (oral) during the 26th Diabetes Poland Congress, and are under review in the Diabetes Technology and Therapeutics.

IV. Initiated projects during Fellowship support: Spatial transcriptomics of pancreatic islets in T1D prevention treatment strategies

In collaboration with Prof. Chantal Mathieu's group at KU Leuven, I initiated exploratory analyses of spatial transcriptomic datasets from pancreatic islets in experimental models of T1D. This work aims to characterize immune–islet interactions and test therapeutic strategies. These analyses extend the fellowship focus from metabolic memory toward mechanistic insights into T1D onset and preventive strategies, establishing a foundation for my planned research visit to KU Leuven, funded by national grants and fellowships (FNP START and NCN-NAWA PRELUDIUM BIS 4).

V. Current status of research work (January – September 2025)

During this reporting period, I have continued to implement the fellowship project and its associated studies, achieving several important milestones.

Ethical approval and insurance for the prospective recruitment study were secured from the Bioethics Committee at the Medical University of Lodz (RNN/236/23/KE). Fellowship funding supported the introduction of minimally invasive TAP microneedle blood sampling, improving feasibility in pediatric participants.

Progress to date:

- Prospective recruitment and follow-up of children with T1D is ongoing, with paired sample collections already underway.
- Laboratory pipelines for DNA methylation assays (Illumina EPIC arrays) have been optimized, with pilot assays completed.
- A meta-analysis of public methylation datasets was finalized (8 cohorts, ~2300 participants), with findings accepted for presentation at ISPAD 2025 (poster).
- Epidemiological manuscripts were submitted/published: (i) 40-year incidence trends of T1D in Central Poland (Hormone Research in Paediatrics, April 2025), (ii) national study on DKA at T1D onset (oral presentation accepted at European Academy of Pediatrics 2025, publication in preparation).
- Studies on insulin pump disconnection protocols during exercise were presented at national conferences, with the first publication published (Hormone Research in

Paediatrics, October 2024) and the other at the Minor Revision stage (Diabetes Technology and Therapeutics).

- A collaboration on spatial transcriptomics with KU Leuven has been initiated, with plans for an extended research visit and secured funding.

Challenges:

- Recruitment pace is limited by participant availability and the need for repeated follow-up visits; this is being mitigated by introducing the TAP micro-sampling method and expanding auxiliary cohorts.
- Large-scale EWAS and ATAC-seq analyses are technically demanding and require careful batch correction; work is ongoing to ensure reproducibility.
- Balancing multiple collaborative projects and dissemination activities with primary recruitment has required careful prioritization.

Despite these challenges, the project remains on schedule, with its outputs having already been presented at international conferences, published or submitted for publication in high-impact journals, and further expanded through international collaborations.

VI. References:

1. Szadkowska A, Pietrzak I, Michalak A, Chrzanowski J, Zmysłowska A, Hogendorf A, Baranowska-Jaźwiecka A, Kuśmierczyk-Kozieł H, Olejniczak A, Dynowska A, Kordialik A, Fendler W, Mianowska B. Increasing Contribution of Adolescent Type 1 Diabetes Drives Incidence Rates in Poland: A 40-Year-Long Observational Study. Horm Res Paediatr. 2025 Apr 17:1-8. doi: 10.1159/000545304.

2. Gawrecki A, Chrzanowski J, Michalak A, Fendler W, Cohen O, Szadkowska A. Novel Protocol for the Use of Advanced Hybrid Closed-Loop System in Adolescents Engaged in Contact Sports. Horm Res Paediatr. 2024 Oct 25:1-11. doi: 10.1159/000542204.

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