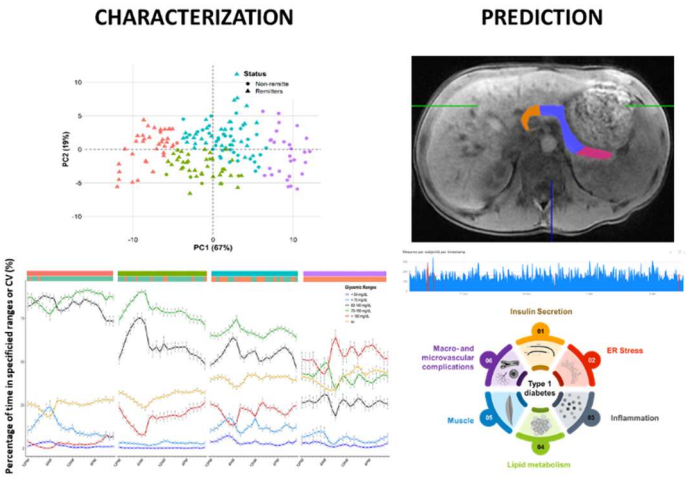

DEEP CHARACTERIZATION AND IDENTIFICATION OF NEW BIOMARKERS OF PARTIAL REMISSION IN PEDIATRIC PATIENTS WITH NEW-ONSET TYPE 1 DIABETES.

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« La pierre n'a point d'espoir d'être autre chose qu'une pierre.
Mais, de collaborer, elle s'assemble et devient temple. »
ANTOINE DE SAINT-EXUPÉRY

La logique vous conduira d'un point A à un point B.
L'imagination et l'audace vous conduiront où vous le désirez.
ALBERT EINSTEIN

Great discoveries and improvements invariably involve the co-operation of many minds. I may be given credit for having blazed the trail but when I look at the subsequent developments I feel the credit is due to others than myself.
ALEXANDER GRAHAM BELL

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« Nous ne faisons pas de nouvelles rencontres par accident. Elles sont destinées à croiser notre chemin pour une raison ». Antoine, j'ai été ravi que les nôtres se soient croisés. Nous partageons tous les deux ce goût pour le désordre, toi encore un peu plus que moi, au grand dam des autres (désolé

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Durant ce marathon, d'autres nous ont quittés. Samuel et Néné, mes pensées sont dirigées vers vous.

SUMMARY

Type 1 diabetes (T1D) is one of the most common autoimmune diseases in children without any current possibilities for curative treatment. While most patients with T1D show a residual β-cell function at diagnosis, current therapies aiming to prevent further loss failed to fully block the ongoing autoimmune attack. Recent evidence suggests disease patchiness and lack of reliable β-cell function and stratification biomarkers as major hurdles for prevention trials. Our research team is investigating different types of pediatric diabetes and participates in T1D prevention protocols. In the present work, we thoroughly characterized disease heterogeneity during the first year of T1D in a multicentric prospective pediatric cohort (i.e., DIATAG) and challenged the current dichotomic definition of partial remission (PR). Furthermore, we identified specific early morning glycemic patterns as new minimal invasive markers of PR and β-cell function. In an attempt to better stratify patients from clinical onset, we found new predictive markers of PR occurrence and glucose homeostasis when analyzing plasmatic proteins and magnetic resonance imaging parameters at diagnosis. Finally, we showed that both occurrence and duration of PR exerted a positive residual effect on short-term post-PR glucose homeostasis. Altogether, this work provides new clues on PR and additional characteristics of pancreatic exocrine alterations in T1D. Also, it highlights new age-related differences in patients with T1D and further supports the need for the inclusion of the pubertal status in trial subanalyses. Lastly, the integration of specific continuous glucose monitoring metrics as new outcomes for prevention trials may improve the accuracy of treatment response evaluation.

ABBREVIATIONS

ACN, Acetonytril

ADA, American diabetes association

ADC, Apparent diffusion coefficient

AGP, Ambulatory glucose profile

AID, Autoimmune islet disease

ATG, Anti-thymocyte globulin

AUC, Area under the curve

BMI, Body mass index

CGM, Continuous glucose monitoring

CI, Confidence interval

$CPEP_{BASAL}$, Fasting C-peptide

$CPEP_{EST}$, Estimated C-peptide

$CPEP_{STIM}$, Stimulated C-peptide

CUSL, Cliniques universitaires Saint-Luc

CV, Coefficient of variation

DIATAG, DIAbetes TAGging

DKA, Diabetic keto-acidosis

DWI, Diffusion weighted imaging

ER, Endothelial reticulum

FC, Fold change

FCP, Fasting C-peptide

FDR, False discovery rate

FF, Fat fraction

GAD, Glutamic acid decarboxylase

GIH_{54-70}, Grade 1 hypoglycemia

$GIIH_{<54}$, Grade 2 hypoglycemia

GRS, Genetic risk score

GST, Glucose stimulation test

$GTAA_{1C}$, Glycemic target-adjusted HbA1C score

HbA_{1C}, Glycated hemoglobin level

HILIC, Hydrophilic interaction liquid chromatography

HLA, Human-leukocyte antigen

HPLC, High performance liquid chromatography

IA-2, Insulinoma-associated antigen-2

IAA, Insulin autoantibodies

IAb, Islet cells autoantibodies

$IDAA_{1C}$, Insulin dose-adjusted HbA_{1C}

IQR, Interquartile range

ISPAD, International society for pediatric and adolescent Diabetes

LC-MS, Liquid chromatography-tandem-mass-spectrometry

LH, Luteinizing hormone

MDI, Multiple daily injections

MMTT, Mixed-meal tolerance

MODD, Mean of daily differences

MRI, Magnetic resonance imaging

PBMC, Peripheral blood mononuclear cell

PCA, Principal component analysis

PHH, Post-hypoglycemic hyperglycemia

PI, Pancreas Index

PM, Proteominer

PR, Partial remission

PRM, Parallel reaction monitoring

PV, Pancreas volume

PVR, Pancreas volume ratio

QoL, Quality of life

R, Spearman rho

r^2, Regression coefficient

REDCap, Research electronic data capture

RF, Rando forest

RFE, Recursive feature elimination

SD, Standard deviation

SMBG, Self-monitoring of blood glucose

SNP, Single nucleotid polymorphism

SOP, Standard operating procedures

SVM, Support vector machine

T1D or T1DM, Type 1 diabetes mellitus

T1DE, Type 1 diabetes endotypes

$TAR_{>180}$, Time above the range

$TBR_{<70}$, Time below the range

TCR, T-cell receptor

TDD, Total insulin daily dose

TFA, Trifluoroacetic acid

$TIR_{70\text{-}180}$, Time in range

$TIT_{63\text{-}140}$, Time in target

TMT, Tandem mass tags

Treg, T-regulatory cells

Zn-T8, Zinc transporter-8

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1 Diabetes: a public healthcare issue with major unmet needs

Diabetes defines a group of metabolic diseases characterized by an inadequacy between endogenous insulin secretion and individual insulin needs, resulting in a rise in blood glucose levels called hyperglycemia. A hundred years ago, the discovery of animal insulins as a substitution therapy changed the medical perspective of patients with diabetes (1) but also uncovered the development of long-term complications such as blindness or kidney insufficiency (2). In the following decades, researchers categorized diabetes into multiple subtypes (e.g., Type 1-3, Maturity-onset diabetes of the young, Latent autoimmune diabetes in adults, lipodystrophies, etc.) (3) with type 2 and type 1 diabetes being the most prevalent groups (i.e., respectively 90 and a bit less than 10% of patients with diabetes) (4–7). The better understanding of underlying disease mechanisms led to the development of medical drugs targeting distinct or common pathways to different diabetes subtypes (e.g., glucose urinary excretion, insulinoresistance, incretins) (8). In that regard, treatment of type 1 diabetes (T1D) remains unsatisfactory as there are currently no approved medications affecting disease progression. Over the last decades, groundbreaking discoveries were made in the management of diabetes (e.g., insulin delivery devices and analogs, glucose monitoring) and that meant for patients an improvement in glucose homeostasis, an increase in quality of life (QoL), and a reduction of diabetes-associated complications (2). Nonetheless, both the incidence and prevalence of diabetes (including T1D) increase worldwide concurring with a global surge of patients with

diabetes-related comorbidities and mortality, and major healthcare expenses. In this context, diabetes research is a matter of public importance. In my thesis, I will focus my research on a better characterization of the evolution of residual β-cell function shortly after clinical diagnosis. I hope that our results will shed new light on the heterogeneity of β-cell function evolution during the first year after diagnosis.

1.1 Type 1 diabetes, an unsolved riddle for more than a century

1.1.1 What stands behind the term type 1 diabetes?

While first reports of diabetes and its symptomatology date back to more than two thousand years ago (9), the groundwork for the distinction between "insulin-insensitive" and "insulin-sensitive" types was performed by Himsworth less than a hundred years ago (10). Since then, current entity called Type 1 diabetes (T1D) was referred to by different terms including "juvenile-onset" or "insulin-dependent" diabetes and characterized from other diabetes by distinct histological and immunological features (i.e., discovery of islet cells autoantibodies [IAb] and Human-Leukocyte antigen [HLA] predispositions) (11,12). Finally, in 1979, a large consortium of diabetes experts classified diabetes mellitus subtypes and attributed his final name T1D (3). In the eighties, George Eisenbarth developed the first conceptual model for T1D pathogenesis that included four stages distributed within two distinct periods (i.e., presymptomatic and symptomatic) (13). This model is still used nowadays although it was recently given small modifications (14). Over the past decade, evidence of heterogeneity within patients with T1D led researchers to question the actual model and progress toward the endotype paradigm **(see 1.3)** (15).

Currently, T1D defines a chronic (auto)immune disorder characterized by the destruction of insulin-secreting β cells located within pancreatic islets of Langerhans. When residual β-cell function drops to 10-20%, patients lose their capacity to maintain their glucose homeostasis with consequent development of continuous hyperglycemia and onset of clinical symptoms (16). If insulinopenia is very severe, patients might develop an episode of ketoacidosis (i.e., acidification of the blood due to abnormally high production of ketones bodies) that is carved with increased mortality and morbidity, especially in countries with low-to-middle incomes or reduced access to the healthcare system (17–19).

After clinical onset, administration of exogenous insulin is life-saving for patients with T1D as it globally restores glucose homeostasis. Nonetheless, current substitution therapy remains a life-long treatment for patients mostly characterized by multiple daily injections (MDI) of insulin that come along with increased burden due to continuous strict monitoring of their glucose level, healthy diet, regular practice of sports and hypoglycemia management (20,21). Despite major advances in insulin substitution therapies and glucose monitoring strategies (8), most patients struggle to achieve the targets of glucose homeostasis (22,23). Occurrence of prolonged hyperglycemia, numerous hypoglycemia episodes and glucose variability lead to a consequent loss of QoL and the development of multiple short and long-term complications (i.e., hypoglycemia, diabetic ketoacidosis [DKA], chronic micro- and macrovascular complications, death) (2,19,24–26). Lastly, particularly when diagnosed during childhood, T1D leads to increased stress in the family (including the fear of hypoglycemia) and

imposes a financial burden, especially in countries without homogeneous healthcare protection (e.g., USA). Altogether, these observations highlight the complexity of disease management and sustain the need for treatment alternatives (24).

1.1.2 T1D, an unraveled rise of incidence for more than 20 years

T1D is one of the most common metabolic conditions in childhood peaking in the pubertal years with more than 128000 new cases worldwide diagnosed annually in individuals below 20 years (27,28). Incidence of T1D varies among countries, ranging from 1.3 to more than 60 per 100000 per year (18). In Europe, the mean T1D incidence is 25.6 per 100.000 per year, peaking at more than 60 per 100000 in northern countries (i.e. Finland, Norway and Sweden) (18,29). Belgium is currently positioned in the lower half of the European ranking (i.e., 18 per 100000 person-years), representing a total of approximately 650 patients with new-onset T1D below 40 years of age (half aged under 15 years) (29). In total, around 0.3% of European children aged below 20 years have T1D (18).

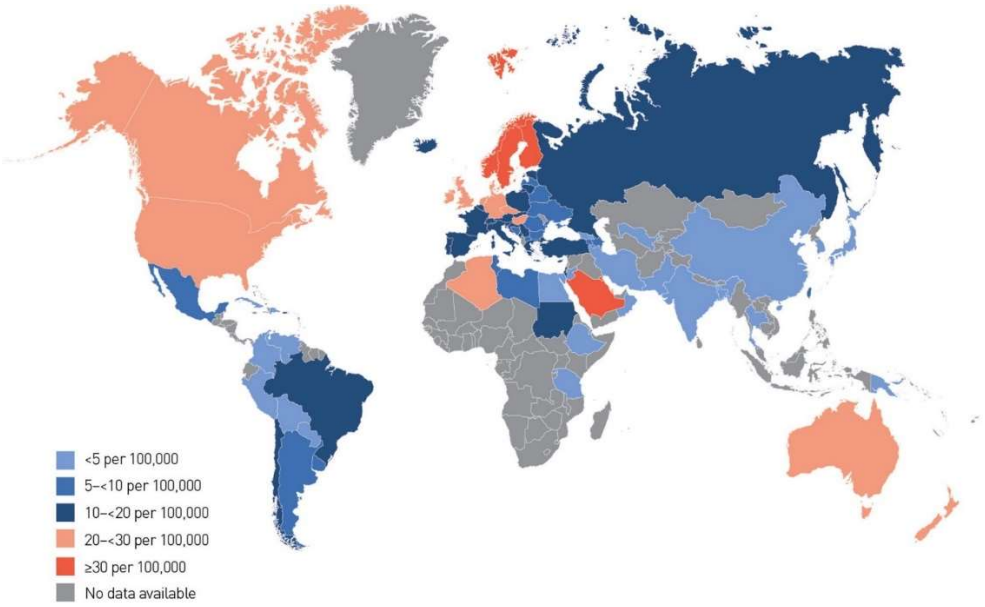

Figure 1: Map of age-sex standardised incidence rates (per 100,000) of T1D in children aged under 15 years. Taken from (18).

Globally, new cases of T1D show an uprising trend worldwide (18,30) (**Fig. 1**). Recent results from the EURODIAB study (i.e., a multicentric European study including 22 countries and with more than 84000 new cases analyzed) demonstrated that incidence of T1D increased by 3.6% annually across all European centers between 1989 and 2013 with no difference observed across genders (29). While EURODIAB showed no difference across age groups, another study suggested a steeper increase in the 0-to-4 years-old age group (31). Interestingly, in the last five years, researchers observed a slowing of this trend in high-incidence countries that currently reached a plateau (32–34). Noteworthy, despite the surge of new-onset T1D, the incidence of asymptomatic patients with T1D within genetically predisposed populations (i.e., multiple IAb+ cohorts) remained stable (35).

While the underlying mechanisms explaining the incidence rise of T1D remain unclear, genetic and environmental factors influence progression towards T1D. Longitudinal birth cohort studies such as The Environmental Determinants of Diabetes in the Young or the Type 1 Diabetes Prediction and Prevention investigated the associations between environmental determinants (e.g., pregnancy, exposure to virus, modification of microbiome) and the progression to β-cell autoimmunity and overt T1D. Putative causes underlying the surge of T1D cases were recently summarized (27) and could be classified into three groups (**Table 1**). Interestingly, only childhood obesity (in the 1990s and early 2000s) and exposure to enteroviruses (mainly different serotypes of Coxsackie viruses) were associated with the development of autoimmune islet disease (AID) and progression to T1D with low hazard ratios (i.e., maximum of 1.4) (36–38). The increasing role of environmental factors in new-onset patients was additionally supported by clinical and epidemiological studies supporting the following evidence: a) emigrating children have the T1D risk of the hosting country (39,40), b) northern European countries show increased T1D incidence despite adjustment for genetic factors (41) and c) a decrease of high-risk HLA proportion in new-onset T1D patients is currently observed (42–44).

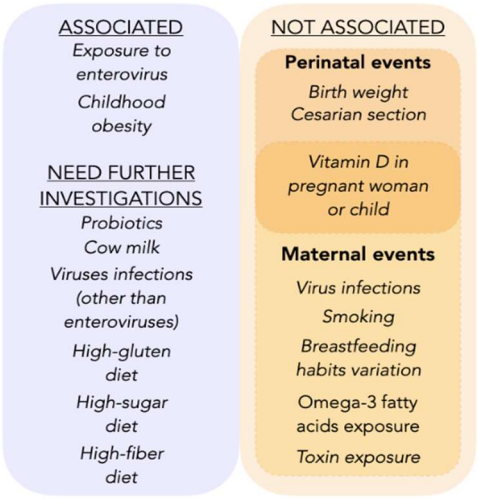

Table 1: Environmental factors and their association with the development of T1D. Based on (27).

While genetic changes alone may not explain such a rise in T1D cases in short time frame, both increases of lower-risk HLA and variants with small effect sizes incidence may participate to the latter (43,45–47). As discussed below, an increasing number of gene variants is being identified using genome-wide associations studies as larger cohorts become analyzed (e.g., meta-analysis) (46,48). Nonetheless, about 20% of T1D inheritability remains unknown and may be explained by the presence of genetic variants with small effect sizes (47,49). These variants may together affect the risk of developing the disease and participate in the increase of T1D (46,50). Linking both hereditable factors and environment, epigenetic changes may also modulate gene expressions and play a role in the susceptibility to develop T1D.

Altogether, these findings suggest that the impact of each environmental factor varies according to genetic susceptibility of the patient (including epigenetic changes) and factor-related time-exposure pattern

(i.e., stage of the disease) (15,51,52). Following this rationale, a recent study proposed that exposure to a minimum of three patient-specific environmental triggers may modulate the penetrance of T1D within genetically-predisposed children and participate in the rapid rise of the disease (15,27). While integration of T1D heterogeneity may improve the efficiency of early prevention studies, identification of these individual factors remains challenging as T1D is asymptomatic for years.

1.1.3 T1D physiopathology: a complex interplay between genes, immunity, β cell, exocrine pancreas and the environment.

Following previous assertions, it has been widely assumed that "(auto)immune attack against β cells occurs in genetically susceptible patients exposed to specific environmental triggers". Recent studies qualified the statement and suggested that both the rupture of immune tolerance and the dysfunction of β cells (e.g., stress response, immunogenicity) are needed to initiate the autoreactive loop leading to the destruction of insulin-secreting cells (53–56). In this subsection, we will summarize most recent evidence about T1D physiopathology and discuss the interplay between genetics, environment, immunity, susceptibility of the β cells and involvement of exocrine pancreas in the initiation of β-cell inflammation and the progression to patent T1D. Further integration of all these facets, at the individual level, is crucial to understand the clinical heterogeneity (discussed in another subsection) and develop T1D prevention therapies (**Fig. 2**)**.**

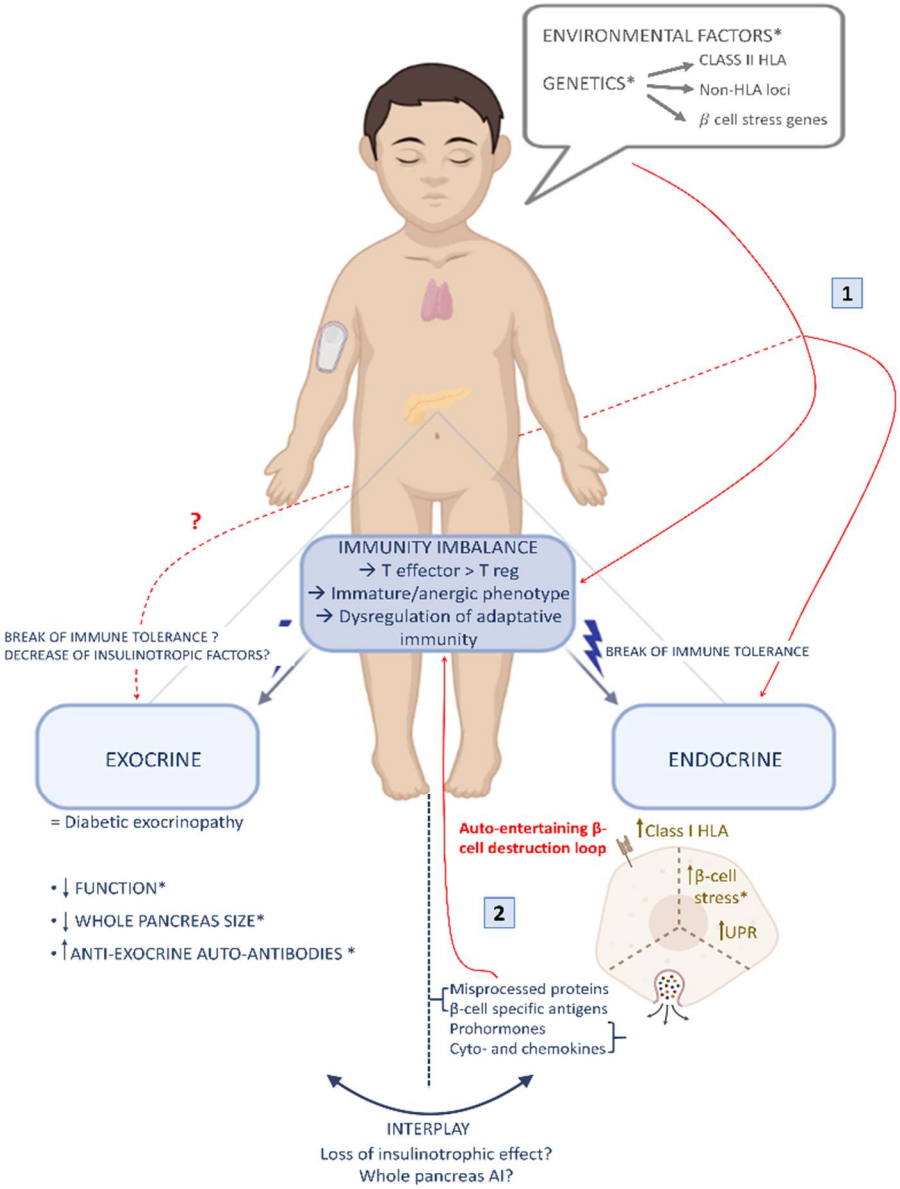

Figure 2: Schematic overview of T1D physiopathology. T1D develops in genetically-predisposed patients exposed to specific environmental factors. Progressive destruction of β-cell mass results from an interplay between genetics, immunity imbalance, β-cell intrinsic vulnerability and the exocrine pancreas.

**= Patient-specific factors, T1D = type 1 diabetes. 1= Initial mechanism(s) leading to β cell injury (i.e., alteration of exocrine cells, immunity disbalance, β cell vulnerability). 2= Auto reactive loop between β cells and immunity*

1.1.3.1 The role of HLA, non-HLA and reticulo-endoplasmic stress genes in T1D susceptibility

As in most autoimmune diseases, less than 10% of patients with T1D have an affected first-degree relative. Nevertheless, the substantial heritable component of T1D is clear: monozygotic twins have approximately 30-50% of concordance while sibling or dizygotic twins have approximately 5-10% chance of developing T1D. Though only a very low number of twins was studied, one study observed a rise of concordance to 85% in monozygotic twins with young-onset T1D (57) and support the preponderant role of genetic background in children below 7 years of age (56,58,59).

T1D is currently considered an oligogenic disease in which 50% of the genetic risk is conferred by class II HLA alleles. Association between diabetes and HLA dates back to five decades ago (60) despite being only recently validated for T1D by genome-wide associations studies (61–63). Interestingly, DR-DQ haplotypes of class II HLA showed some specificity with HLA DR3/DR4-DQ8 and DR4-DQ8/DR4-DQ8 bearing the strongest associations (64). Currently, more than 60 additional non-HLA loci or single nucleotide polymorphisms (SNP) were associated with T1D with most being located in gene regulatory regions (62,65). These variants can be categorized into three majors subgroups according to their relative implication in key elements of T1D physiopathology (i.e., immunity [e.g. INS, cytotoxic T-Lymphocyte Associated Protein 4 (CTLA4), protein tyrosine phosphatase non-receptor type 22 (PTPN22)], inflammation [e.g., interleukin 2 Receptor Subunit Alpha (IL2RA)], and the β-cell vulnerability [e.g., interferon pathway

(melanoma differentiation-associated protein 5 (MDA5), tyrosine kinase 2 (TYK2))]). More recently, risk of developing multiple IAb was also linked to class II HLA and other non-HLA loci and supported the role of genetic background from the very early moments of T1D development (66).

These observations together lead to the development of T1D multi-loci genetic risk scores (GRSs). These GRSs aggregate the risk conferred by the presence or absence of multiple SNPs into a continuous scale to identify individuals at risk to develop β-cell autoimmunity (58,66,67). Identification of this high genetic risk population allowed the longitudinal evaluation of cohorts from birth to patent T1D (68), a decrease of DKA at clinical onset (69,70) and the inclusion of high-risk patients in primary prevention trials (**see 1.5**). Besides being used as a screening tool to identify predisposed patients, GRSs in combination with clinical features of the patients demonstrated to be powerful in both predicting the progression from autoimmune islet disease to T1D (i.e., AUC > 0.8 at 3 years) (58,71) or classifying types of diabetes (i.e., T1D *vs* other subtypes) (72,73).

1.1.3.2 Innate and adaptative immunity participate in the self-amplification loop concurring with the demise of β cells

Supporting the preponderant role of immune-related genes in T1D development, it is commonly accepted that both innate and adaptative immunity participate in the autoimmune attack against insulin-secreting cells (**Fig. 3**). Environmental factors, in combination or not with the intrinsic fragility of the β cell (see 1.1.3.3), trigger a local inflammation called insulitis. Inflammation of the β cells leads to increased cellular stress (i.e., oxidative

and reticulo-endoplasmic [RE] stress) and overexpression of their surface class I HLA, increasing together their immunovisibility and presentation of (neo)antigens (see 1.1.3). These peptides are recognized by antigen-presenting cells and presented in the pancreatic lymph nodes to autoreactive $CD4^+$ T-cells. Recognition of antigens by these lymphocytes showing an incomplete thymic maturation (e.g. low specificity of TCR, reduced production of tissue-specific antigen in the thymus) further induces their lineage commitment. Differentiated autoimmune CD4+ cells recruit and activate autoreactive CD8+ and B lymphocytes (production of autoantibodies against islet-cell components). These activated CD8+ cytotoxic T-cells migrate to the pancreas and destroy the insulin-secreting cells with a consequent release of β-cell-specific antigens and the production of a pro-inflammatory environment (74). These cytokines and chemokines participate in the recruitment of humoral cells (e.g., macrophages) and accelerate β-cell demise. While peripheral mechanisms of immunotolerance usually control autoreactive lymphocytes in healthy individuals, regulatory T-cell (Treg) of patients with T1D demonstrated to be anergic and poorly responsive to IL-2 participating in the break of immune tolerance (75) .

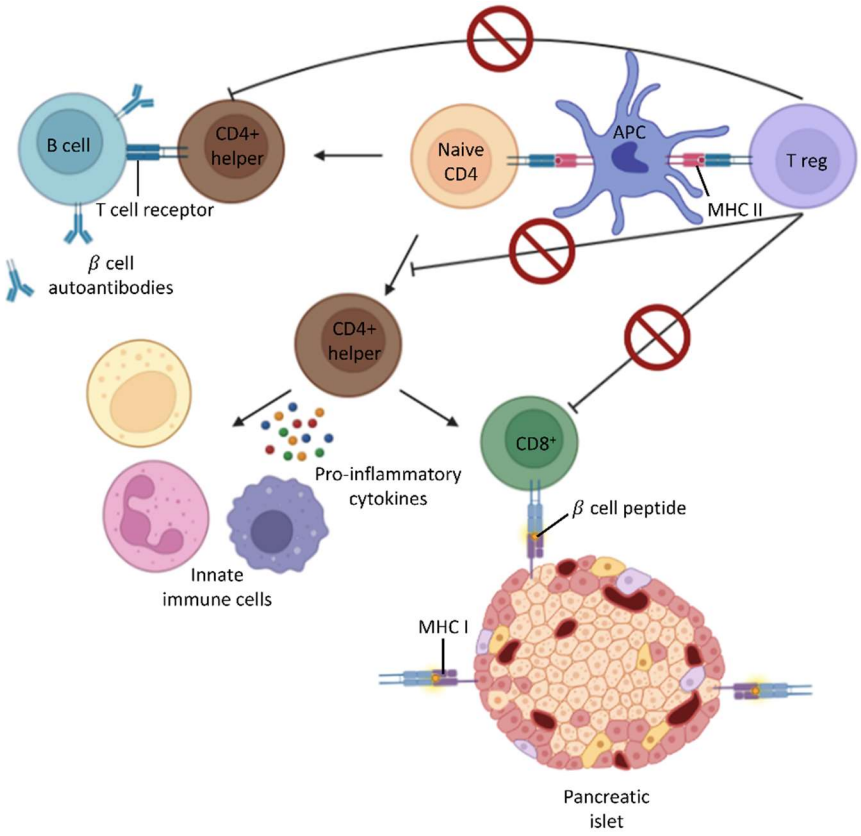

Figure 3: Immunopathogenesis of T1D - Interactions between (auto)immunity and β cell. Environmental factors may trigger β-cell inflammation in genetically predisposed patients and expression of self-antigens. APCs present those β-cell antigens to autoreactive T cells that showed immature thymic maturation phenotype. The activated autoreactive CD4 T cells activate autoreactive CD8 T cells and β cells. Production of proinflammatory cytokines by both the β cells and activated CD4+ lymphocytes participate in the recruitment of innate immune cells. APCs= antigen-presenting cells

Adapted from DiMeglio et al. (16) *and Master thesis of Julie Lemmer.*

Several observations in patients with T1D supported this immune cascade (55) (genetic background was previously discussed). Firstly, the presence of circulating autoantibodies targeting islets-specific antigens (i.e., anti- glutamic acid decarboxylase [GAD], islet antigen 2 [IA-2], anti-Insulin, zinc transporter 8 [ZnT8], tetraspanin-7) currently remains the most reliable biomarker of both the autoimmune activity and the progression to patent T1D although their pathogenic role is not established (76). Secondly, studies demonstrated the presence of autoreactive T-cells (i.e., $CD4^+$ and $CD8^+$) both in the bloodstream (77) and the pancreatic tissue (77,78) of patients with T1D. A study of the on-site peptidome showed that these cells reacted against known β-cell antigens such as GAD, proinsulin, or modified autoantigens (74). Further supporting these assertions, a single case report showed that the transplantation of non-T-depleted bone marrow of a patient with T1D to his naïve twin led to the clinical onset of T1D in the latter patient (79).

Nonetheless, as recently reviewed by Roep et al (55), gaps remain if considering the immune system as the only cause of the demise of β cells. First, only 10% of patients having a single anti-islet autoantibody will evolve to patent T1D (80). Second, as highlighted in a recent review by Mallone & Eizirik (56), $CD8^+$ T-cells reacting against islet components are present in similar frequencies in the bloodstream of healthy and T1D patients (77,81,82). Conversely, these autoreactive cells were enriched in the pancreas of patients with T1D suggesting local homing factors (77,83). Third, immunomodulative therapies showed heterogeneous and short-lasting effects on the preservation of β-cell residual secretion (69). Finally,

histological studies of the pancreas of new-onset patients with T1D showed diffuse β-cell stress with only some islets presenting the characteristics of insulitis (84,85). The immune system may thus participate in the death but also enhance cellular stress of β cells, self-amplifying the immune attack and precipitating their demise (55,56). Importantly, while the role of autoimmunity appears to be more important in children with young-onset T1D (86,87), the balance between both autoimmune response and β-cell stress remains likely patient-specific (see 1.3).

1.1.3.3 β cells participate and enhance their own destruction

As initially proposed by Bottazo in 1986 (88) and discussed here above, the progressive destruction of β cells relies on a complex interplay between the immune system and the susceptibility of β cells to oxidative and cellular stress (e.g., biosynthetic). When we ingest food, insulin-secreting cells need to "instantly" increase by up to 50-fold the rate of (pro)insulin production. This high level of metabolic demand induces an increase in chaperones' production and unfolded protein response to reduce cellular stress (89). Nonetheless, low expression of detoxification enzymes and anti-apoptotic proteins in β cells may limit these protective mechanisms (90,91). When overwhelmed, these processes progressively drive β cells to increased immunogenicity, exhaustion, and finally apoptosis (56). The threshold of β-cell stress tolerance may be specific to each individual (i.e., genetic predisposition) and influenced by (micro)environmental factors (i.e., virus, hyperglycemia, inflammation, vascularization). These observations provide potential mechanisms in β-cell diseases such as diabetes.

In pro-inflammatory conditions such as T1D, β cells fan the flame by upregulating specific surface markers (e.g., receptors to viruses (92), class I (93) and class II (94) HLA), secreting attracting chemokines (95), increasing their response to cellular stress (86,96–98) and accumulating immunogenic misprocessed proteins. Indeed, stressed β cells generate neo-epitopes that by-pass both regulatory cells repertoire and thymic negative selection (i.e., misfolding, generation of neoantigens, posttranslational-modifications, defective ribosomal products). Furthermore, stressed β cells abnormally process prohormones (e.g., proinsulin, islet amyloid polypeptide [$IAPP_{1-48}$]) which revealed to be major insulin-secreting-cells antigens and accumulated before clinical onset (99,100). The progressive increase of prohormone/mature hormone ratio across T1D stages (99) supports that the decline of β-cell mass intensifies the cellular stress of the remaining cells. Altogether, these data suggest that β cells act as amplifiers of the autoimmune response (86).

1.1.3.4 Type 1 diabetes is a whole pancreatic disease

Another emerging concept supports T1D as a whole pancreatic disease (101). Indeed, the pancreas is an amphicrine gland with an endocrine (islet cells) and an exocrine (acinar cells) part whose functions converge into nutrient assimilation and glucose homeostasis. Although their respective roles differ, acinar cells are interconnected with β cells (e.g., β-cell neogenesis from ductal cells) and altered in patients with diabetes (102,103). While first description of pancreatic exocrine tissue in patients

with T1D dates back to several decades (104,105), the "β-cell specific" paradigm set aside these abnormalities for a long time. About a decade ago, researchers regained interest in the exocrine compartment and deepened the characterization of exocrine tissue modifications in diabetes mellitus (T1D and others) at multiple levels (i.e., transcriptomic, proteomic, histology, cytology, clinically) (103,106–108). These observations jointly lead to the description of a novel histologic entity called diabetic exocrine pancreatopathy and defined by Mohapatrah as a "*moderate-to-severe subclinical pancreatic fibrosis associated with a modest exocrine dysfunction occurring in the absence of clinical or histopathological evidence of chronic pancreatitis"* (109).

In patients with T1D, the hallmark of exocrine pancreatopathy corresponds to global atrophy of the pancreas. These patients show a reduction of approximately 50% of their pancreatic volume, while islet cells represent less than 2% of pancreatic weight. Notably, this atrophy is present from pre-symptomatic stages (110) and progresses during the 5 years following T1D onset (111). Exocrine tissue of patients with T1D is also characterized by structural abnormalities such as abnormal acinar cells (number, size, morphology) (111,112), fibrosis (113), vascular changes (114) and pan-immune infiltration (i.e., presence of CD4+, CD8+, $CD11c^+$ cells, neutrophils, and increased complement activation) (115). Deep profiling of the exocrine tissue (116–118) further revealed a dysregulation of pancreatic exocrine function (119,120), an increase of complement cascades activation (117,119), the presence of neutrophil extracellular traps (117) and intermediate cells (i.e., exhibiting both endo- and exocrine granules)

(117,121). Conversely, another study conducted on the pancreas of a living donor with new-onset T1D showed downregulation of immune, inflammation, and β-cell regeneration genes in whole-pancreas transcriptome analysis (120). Finally, these structural abnormalities were associated with biological exocrine insufficiency in up to 80% of patients with T1D (e.g., decreased levels of fecal elastase 1, serum trypsinogen and serum lipase) though remaining mostly subclinical (122,123). Interestingly, IAb+ patients showed a progressive and heterogeneous decrease of circulating exocrine function markers before clinical onset (124).

Mechanisms and temporality of exocrine pancreas damage remain unclear (i.e., before, concomitantly, or after the destruction of β cells). As recently suggested by Vecchio (125) and Foster (122), whole pancreatic inflammation and/or immune attack may trigger a concomitant loss of both acinar and insulin-secreting cells in a subset of patients. Firstly, 20-70% of patients with T1D have circulating autoantibodies against the exocrine tissue (e.g., anti-bile salt-dependent lipase, anti-pancreatic cytokeratin, anti-lactoferrin) (117,126,127). Secondly, sporadic case descriptions suggested that the latter autoimmunity may appear early in the disease process as some first-degree relatives displayed circulating autoantibodies against bile salt-dependent lipase prior IAb seroconversion (128). Thirdly, as previously discussed, some patients with T1D have an increased genetic susceptibility to inflammation and/or oxidative stress (e.g., SNPs in GLIS Family Zinc Finger 3 [GLIS3], X-Ray Repair Cross Complementing 4 [XRCC4]). These patients might be more prone to experience whole pancreatic inflammation with more pronounced and continuous damage to the β cells due to their higher

intrinsic fragility (86). Recent evidence linked both the exocrine and endocrine compartments as on the one hand combination of serum markers of exocrine function predicted both the pancreas volume and stages of T1D (129) and on the other hand, patients with T1D showed an enrichment of sequence polymorphism in regulatory elements of exocrine cells (130). However, whether the exocrine pancreas in patients with T1D shows specific topography in its atrophy and exact relationships with the endocrine function remains poorly described.

We may summarize T1D as a (pan)immune-mediated disease that develops in genetically predisposed children exposed to proinflammatory environmental factors. These elements together trigger the initial insulitis and the (auto)immune reaction. The self-entertaining loop concurring to the destruction of most β cells relies on an interplay between the immune system, the intrinsic vulnerability of β cells, and the exocrine tissue in which each factor's weight depends on both the individual characteristics of the patient and the stage of the disease.

1.2 Staging and management of type 1 diabetes

Type 1 diabetes is a long-evolving disease that starts from months to years before the clinical onset. About 30 years ago, Georges Eisenbarth first proposed that an asymptomatic period preceded the clinical onset of T1D (13). From that moment, international diabetes societies (e.g., International Society for Pediatric and Adolescent Diabetes [ISPAD], American Diabetes Association [ADA]) divided T1D into three different stages characterized by

specific combinations of circulating insulin autoantibodies, levels of glucose intolerance, and the presence of hyperglycemia-related symptoms (i.e., polyuria [excessive urination], polydipsia [excessive drinking] and weight loss) (14) (**Table 2, Fig. 4**). Notably, this classification was strengthened by the description of stage-specific histopathological features (i.e., β-cell loss, insulitis, HLA-I expressing, viral markers, and immune infiltration) (131) (**Table 2**). Nonetheless, current markers fail to identify patients that will progress through the different stages and finally to overt T1D.

Recently, researchers suggested dividing T1D into two distinct entities: AID that relates to the primary disease (i.e., stages 1 and 2) and overt T1D (i.e., stages 3 and 4) that corresponds to the end-stage of AID. This second definition encompasses current T1D staging as AID includes patients with only one IAb+ and allows the stratification between transient [IAb+ reverters], less aggressive [1 persistent IAb], or more aggressive [>2 IAbs] AID (132). In the latter model, environmental or genetic factors act as promoters and/or accelerators of the evolution of AID (e.g., virus infection, non-HLA genes, characteristics of (auto)immune response). While refining current definition of T1D, the concept of AID needs further clinical investigations and validations (132).

Classification in T1D					
Symptoms of the patient	Asymptomatic			Diabetes triade	
Entities in Diabetes	Autoimmune Islet Disease (AID)			Overt T1D	
Stages of diabetes	0	1	2	3	4
Characteristic of the patient					
Presence of IAb	Yes, 1 IAb	>2 IAb	>2 IAbs	At least 1 IAb	At least 1 IAb
Presence of dysglycemia	No	No	Near-normal glycemic values	Above diabetes metabolic criteria	Above diabetes metabolic criteria
Diabetes-related symptoms	No	No	No	Yes	Yes
Biological and histological features					
Islets					
Insulitis (immune infiltration)*	-	+	++	+++	++/+
B-cell loss*	-	+	++	+++	+++
Expression of class-I HLA*	Rare	+	++	+++	++
Witness of virus infection*	+/-	+	++	+++	++
Residual C-peptide secretion	Normal	-	-/- - (12 months before onset)	+/-/--	--/---
Markers of B-cell stress	+/-	+	++	+++	++
Exocrine pancreas					
Exocrine enzymes (i.e. FE-1, trypsine or lipase)	Normal	?	-	--	--
Pancreas volume	- (maybe little decrease in FDR)	+/-	++	+++	+++ (stable >5y after onset)
Acinar atrophy	Normal	Normal	Normal till close to T1D onset	decreased number	decreased number
Immune infiltration	?	+	+	+++	+

Table 2: Clinical and biological characteristics of type 1 diabetes according to disease stages**.** *Adapted from Richardson et al* (131).

1.2.1 The challenges of the presymptomatic period

The presymptomatic period englobes the two first stages of T1D and corresponds to clinically asymptomatic patients with two or more IAb+ that experience (i.e. stage 2) or not (i.e. stage 1) episodes of dysglycemia (see below for diabetes diagnosis criteria) (**Table 2**). After the first autoantibody appears (i.e., usually before 2 years of age), the progression to multiple autoantibodies relies on a combination of genetic and environmental accelerators (see 1.1.3). Even though the temporality of progression to overt T1D is difficult to predict, young children with more than two antibodies have a risk of 84% to develop T1D before the age of 18 years old (80).

While being clinically asymptomatic, patients with stage 2 T1D already have multiple pancreatic structural (e.g., reduced pancreas volume, whole-pancreas immune infiltration, insulitis, inflammation) (83,110,115,133) and biological abnormalities (e.g., increased β-cell stress, reduced exocrine function, transient dysglycemia) (54,124,134). Moreover, a recent study showed that these patients also displayed measurable β-cell dysfunction more than 5 years before the clinical onset (135). Interestingly, these authors also revealed the existence of two distinct and sequential dynamics in the decline of residual β-cell secretion. Patients first demonstrated a relative stability of metabolic measures for many years (i.e., very slow worsening of the metabolic indices) before experiencing an abrupt fall in the 6 to 12 months preceding T1D onset (135,136). While both progressors and non-progressors observed similar patterns for the first part, only progressors showed rapid metabolic deterioration to the clinical onset of T1D (135,137). While factors underlying this binary evolution are unclear, we might either suspect an immunological burst, an acute collapse of the β-cells (i.e., exceeding the tolerance threshold), or a combination of both events (138).

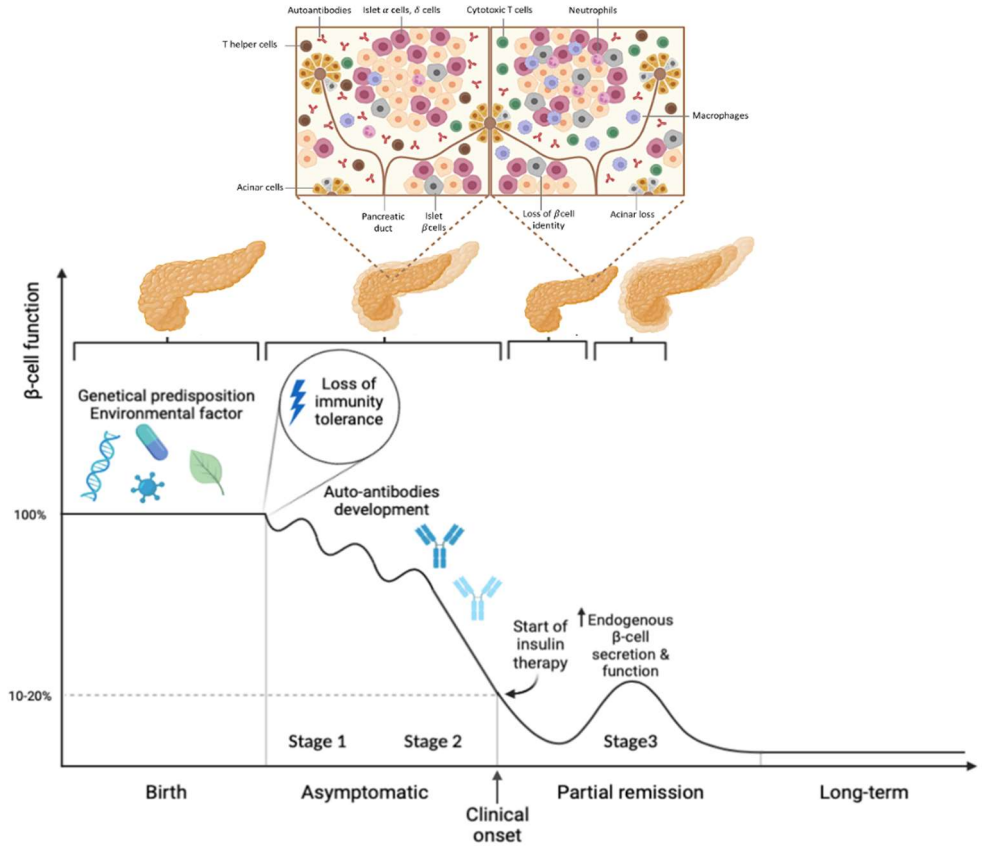

Figure 4: Natural history of T1D. Immune tolerance breaks down in genetically predisposed individuals following exposure to environmental factors. Stages 1 and 2 correspond to an asymptomatic period of T1D with the presence of autoantibodies and progressive loss of β-cells. In stage 2, the patient begins to experience phases of asymptomatic dysglycemia. Stage 3 corresponds to the onset of clinical symptoms when 80-90% of β-cells are destroyed. The inserted panels correspond to the evolution of pancreas histology (*upper*) and pancreas volume across T1D stages (*lower*). At early stages of T1D (i.e., 1 and 2), we observe an infiltration of whole pancreas by $CD8^+$, $CD4^+$, macrophages, and neutrophils, and a reduction of secreting β-cell and acinar cell content (*upper left panel*). After onset (*upper right panel*), anergic β cells, macrophages and neutrophils further increase (*grey β cells)* with a reduction of acinar cells number and size. *Adapted from Fonolleda* (139) *and Powers* (140). T1D= type 1 diabetes.

1.2.2 The clinical onset of type 1 diabetes, an end-stage disease step.

Hyperglycemic excursions and hyperglycemia-related symptoms appear when residual β-cell function drops below a certain threshold (i.e., commonly described as 20%). ADA (141) and ISPAD (69) define overt T1D as patients with **at least one IAb** (i.e., anti-GAD65, anti-insulin, anti-IA2, anti-ZnT8 or anti-tetraspanin7) **and metabolic features of diabetes** including:

1. Fasting plasma glucose value ≥126 mg/dL (7 mmol/L)
2. OR random blood glucose ≥200 mg/dL (11.1 mmol/L) **AND** the presence of clinical symptoms of hyperglycemia (i.e. polyuria, polydipsia or weight loss)
3. OR an abnormal oral glucose tolerance test (≥200 mg/dL at 120 minutes)
4. OR HbA1C ≥6.5% (48 mmol/mol).

Diabetes onset during childhood and severe insulinopenia (i.e., decrease of C-peptide) at diagnosis (associated or not with DKA) support the diagnosis of T1D (142). Nonetheless, caution must be taken when classifying patients into diabetes subtypes as 10% of patients with T1D do not have associated IAb+ (143) and approximately 50% of T1D cases occur in adults (144).

At diagnosis of T1D, minimal residual β-cell secretion is observed in the greatest majority of patients with the majority having C-peptide levels above 0.2 pmol/mL (137,145). Nonetheless, as the immune attack persists, residual β-cell mass continues to decrease during the first years after clinical onset (137). This evolution is heterogeneous within patients, which might

partially explain a similar heterogeneity in remission or “honeymoon” period (see 1.3.3) (139,146).

1.3 Heterogeneity in type 1 diabetes leads to clinical translational challenges

Current diagnosis and management of T1D rely on the consideration of T1D as a single nosological entity. However, recent evidence supports the existence of multiple clinical subgroups within patients, recently named “endotypes”(15). The concept of endotype derives from the field of asthma research and underlies that multiple pathways might lead to the development of the same disease phenotype (147). While an array of different mechanisms may converge to the destruction of β cells (55,56), recent evidence suggests the existence of multiple distinct T1D phenotypes (15). Specific biological and histological features were found within the latter population including group-specific genetic predispositions, immune profiles, pancreatic pathologies, kinetics of β-cell demise, ages at clinical onset, response to immunomodulation therapies, and long-term development of vascular complications (140,148). These were recently reviewed by different authors (5,131,140). While some of these differences were previously described in the manuscript, this paragraph aims to synthesize the most recent knowledge regarding the latter with a special focus on the clinical stages of T1D. **Table 3** summarizes most age-related differences.

1.3.1 Disparities are present from the earliest stages of T1D with age-related patterns

Longitudinal birth-cohort studies showed high inter-patient heterogeneity in the initiation of the immune process and the disease progression across T1D stages. Accordingly, the apparition of the first IAb is polarized by the HLA-subtype of the patient where carriers of HLA-DR4 develop anti-IAA and carriers of HLA-DR3 develop anti-GAD (149–151). Moreover, the type of the first IAb also demonstrates an age-specific pattern where anti-IAA peaked in very young patients (i.e., 12 months old) with an incidence rapidly decreasing after 2 years old, and anti-GAD that appeared later in life with a stable incidence (149,150). Linking these seroconversion groups to clinical characteristics, a recent study showed that patients with first-appearing anti-IAA were mainly boys with young-onset T1D and high-risk HLA while patients with first-appearing anti-GAD were mainly girls with older age at onset and a lower risk HLA (151). These autoantibodies groups were also characterized by specific non-HLA loci (152). All these information together support the presence of different phenotypes as from the initiation of insulitis.

Though showing no correlation with the type of first-appearing autoantibody (150), the progression to stages 2 and 3 showed various trajectories associated with distinct features (153). Current studies showed that younger age at seroconversion, shorter time-lapse between single to multiple IAb progression, the titre of autoantibodies, and the type of the second autoantibody (i.e., especially anti-IA2) increased the risk of progression to overt T1D (150,154–156). Interestingly, this progression was

also influenced by the genotype of the patient in an age-dependent manner. While high-risk haplotypes and some minor alleles (i.e., non-HLA loci) conferred a higher risk in both young and old children, others differed between age groups (**Table 3**) (152,154). Corroborating most of these assertions, a recent study identified three IAb trajectories in the progression to T1D that exhibited age, HLA-DR and sex-specific features (153). Fulfilling these observations, patients with stage 2 T1D showed different patterns of C-peptide decline according to their age subgroup (i.e. <11, 11-20, >20) with the youngest having the most abrupt decline (137). Finally, several studies showed diversity in other clinical features including immune profiles (157,158), serum biomarkers (124,159) and pancreas histology (i.e., variable extents of insulitis across the pancreas) (160) in the same presymptomatic window.

1.3.2 Clinical phenotype and evolution surrounding type 1 diabetes onset demonstrate age-related differences

Distinct age-related histological subtypes were recently described at T1D onset by *Leete et al* (161). T1DE1 patients (<7 years old) were characterized by an increased islet infiltration of B-lymphocytes ($CD20_{high}$), decreased β-cell mass, and abnormal co-localization of insulin and proinsulin within the islets (i.e., alteration of prohormone processing). On the contrary, T1DE2 patients (>12 years old) had increased β-cell mass, fewer immune infiltration ($CD20_{low}$), and minimal insulin-proinsulin co-localization (161,162). These immunohistological analyses were supported by distinct clinical characteristics with children below 7 years of age having a higher prevalence of relatives with T1D, stronger effect sizes of T1D risk variants (i.e., HLA and

non-HLA) and increased frequency of multiple autoantibodies at diagnosis (163,164). Disparities were also identified for immune and metabolic markers with the youngest patients showing decreased residual β-cell secretion (137), increased C-peptide:proinsulin ratio (159,161) and specific immune-cell signature (i.e., higher levels of B lymphocytes, lower levels of circulating neutrophils) (165). Finally, our team showed for the first time that children with prepubertal onset of T1D presented increased pancreatic atrophy. These data altogether suggest a more aggressive disease phenotype in children with young-onset, with a preponderant role of autoimmunity and genetic predispositions (56).

After clinical onset of T1D, patients showed high variability in the decline of β-cell residual secretion (137) and the evolution of glucose homeostasis (e.g., glucotypes or PR). New-onset T1D patients showed either fast or slow loss of residual C-peptide which was associated with, for example, different clinical and immune characteristics (e.g., age at onset (146,165,166) and immune signatures (165,167)). Patients may also be categorized according to the early evolution of glucose homeostasis parameters (e.g., HbA_{1C}, total daily dose [TDD], glucose variability index, etc.). On the one hand, distinction between remitters and non-remitters is the most common method (see below). On the other hand, our group expanded the latter definition and identified four distinct glucotypes during the first year of T1D. These were present from three months postdiagnosis with youngest patients distributing in the most dysbalanced glucotypes (i.e., 3 and 4) (168).

Better characterization of T1D heterogeneity may allow early stratification of patients and improve results in residual β-cell prevention trials (see 1.5) (56,72,138,169). In that regard, using a multilevel approach to gathering information from pancreas imaging and evaluation of both the exocrine and endocrine function might allow new insights into the heterogeneous physiopathology of T1D. As an example, a very recent multilevel approach integrated phenotypic, genotypic, and biological markers in patients with new-onset diabetes and identified seven different IAb^+ subgroups at disease onset that demonstrated distinct HbA_{1C} trajectories seven years postdiagnosis (170).

- High heterogeneity in patients with T1D exists from the earliest stages of T1D.
    - Genetic, metabolic, histological, immune and exocrine specificities
- Most of these disparities have an age-related pattern and support a more aggressive disease in children with young-onset (i.e., prepubertal).
    - Identification of T1D endotypes (e.g., T1DE1 and T1DE2)
- Integration of T1D heterogeneity in clinical decision-making may allow better stratification of patients and patient-tailored prevention therapies.

	Initiation of insulitis and seroconversion (stage 0-1)		Disease progression (stage 1-2)		T1D diagnosis and early evolution (end stage 3)	
	Younger	*Older*	*Younger*	*Older*	*Younger (T1DE1)*	*Older (T1DE2)*
Phenotype						
Gender	Young seroconverters --> IAA = boys with young onset	Older seroconverters --> GAD = girls with late onset	Gender may have marginal effect on progression trajectories in a non age-dependent way		Female>male	Male>female
First degree relative	/	/	/	/	Increased incidence of FDR	Decreased incidence of FDR
DKA	/	/	/	/	Non-homogeneous data (increased DKA in young onset or in older onset).	
Genetic						
HLA subtypes	Young seroconverters --> HLA-DR4, ++ high-risk HLA	Older seroconverters --> HLA-DR3, ++ low-risk HLA	High-risk HLA influence progression is non age-dependent (but… more high-risk HLA in young onset)		Increased indicdence of high-risk HLA	Decreased high-risk HLA and increased low-risk HLA
Non-HLA	/	/	Some minor non-HLA loci influence progression in a non age-dependent way (e.g., PTPN22)		Age-dependent influence of INS or others See Non-HLA in **Disease progression column**	
			PTPRK, THEMIS, IAA increase risk of young onset	PFKFB3 increased risk of late onset		
Immuno-metabolic						
Auto-antibodies	Young seroconverters --> ++ anti-IAA, mainly in first 2 years	Older seroconverters --> anti-GAD, stable during life	Young progressors = Increase numer of multiple IAb+, higher title of IAA, younger at seroconversion	Older progressors = Higher title of GAD and ICA, older at seroconversion	Young-onset have a higher incidence of multiple IAb+	Older-onset have higher incidence of GAD positive
Innate immune signature	/	/	Lower circulating neutrophils count is associated to reduced β-cell secretion		Young-onset have lower circulating neutrophils	Older-onset have higher circulating neutrophils
			Weaker association between C-peptide and circulating neutrophils in <5yo	Stronger association between C-peptide and circulating neutrophils in >12yo		
Adaptative immune signature	/	/	/	/	Some immune signatures are non age-depent (e.g., similar T-cell response in young and old)	
					Young-onset have higher circulating B lymphocytes	Older-onset have lower circulating B lymphocytes
β-cell stress	/	/	β-cell ER dysfunction (i.e., circulating PI:C) is increased before clinical onset		Young-onset have increased circulating PI:C ratio	Older-onset have decreased circulating PI:C ratio (but higher than controls)
			Highest PI:C ratio in children <5yo	Lowest ratio PI:C in children >5yo		
β-cell function	/	/	Steeper decline of β-cell function in young children	Slower decline of β-cell function in children >12yo	Faster loss of C-peptide post-diagnosis, decreased incidence of PR, increased in glucotypes 3 and 4	Slower loss of C-peptide post-diagnosis, increased incidence of PR, increased in glucotypes 1 and 2
Histology						
Endocrine pancreas	/	/	Patchy insulitis, influence of age was not studied?		$CD20_{HIGH}$, decreased β-cell mass, high insulin-proinsulin colocalization	$CD20_{LOW}$, increased β-cell mass, low β-cell insulin-proinsulin colocalization
Exocrine pancreas	/	/	Various level of atrophy, influence of age was not studied		Increased atrophy	Atrophy but less than young children

Table 3: Summary of age-related phenotypic, genotypic, immune-metabolic and histologic specificities according to T1D stage. Adapted from Power *et al* (140) and *Battaglia et al.* (15)

1.3.3 *Partial remission, an optimal but poorly characterized and heterogeneous period for initiation of prevention studies*

One specific example of T1D heterogeneity is the occurrence of a spontaneous and transient relapse of T1D in the weeks following the initiation of insulinotherapy. This period, called partial remission (PR) or "honeymoon phase", is characterized by a resurgence of endogenous β-cell function and has a mean duration of 9 months (139). High variability in its prevalence (ranging from 30-80%), duration (i.e., few months to more than a year), and intensity were observed among patients with new-onset T1D (139). For example, 1-3% of new-onset patients experience a complete remission characterized by near-normal glucose control without insulin injections for a couple of weeks (139).

Clinically, PR is illustrated by a composite of low glycemic fluctuations, low insulin daily needs and low HbA_{1C} levels in the context of increased endogenous β-cell secretion (i.e., C-peptide) and reduced insulinoresistance (see below). In that regard, multiple definitions of PR have been proposed including:

A. <u>Definitions based on thresholds of glucose homeostasis-related parameters:</u>
    a. Residual endogenous secretion above 300 pmol/L (171,172)
    b. Low insulin daily needs with TDD below 0.3U/kg/day (173)

c. Combination of low TDD (i.e., <0.5 U/kg/day or <0.3 U/kg/day) and glucose metabolic stability (HbA1C <7.5% or <7%) (174)

B. <u>Definitions based on clinical composite scores (i.e. formula) :</u>

a. The Insulin dose-adjusted HbA1C ($IDAA_{1C}$) which is equal to $HbA_{1C(\%)}$ + (4 × insulin dose (U/kg body weight per 24 h [TDD])) where a score below 9 defines patients undergoing PR (171). **This definition is the most commonly used in the clinical routine and advocated by the ISPAD** (69).

b. The glycemic target-adjusted HbA1C score ($GTAA_{1C}$) that combines HbA_{1C} and the percentage time spent in normoglycemia (i.e. in 70-180 mg/dL range), and is equal to $HbA_{1C(\%)} – (3* [\%\ of\ normoglycemia_{70\text{-}180\ mg/dL}])$. A score below or equal to 4.5 predicts PR. This score was developed by our team and encompasses some limitations of $IDAA_{1C}$ such as misreporting of TDD by the patient and inclusion of continuous glucose monitoring (CGM) data (175).

Presently, the $IDAA_{1C}$ score is considered as the gold standard to define PR (139,176) as it correlated with residual secretion >300pmol/L and was validated in multiple pediatric cohorts (177,178). Nonetheless, some authors (178–180) questioned this definition as $IDAA_{1C}$ score does not take into account insulin sensitivity, gender, or pubertal status at T1D onset. Indeed, these individual factors influenced $IDAA_{1C}$ parameters and may lead

to an underestimation of PR occurrence especially in pubertal girls or during the first 3 months after T1D diagnosis (178). Moreover, as previously mentioned, TDD is dependent on the patient's compliance (i.e., reporting and carbohydrate intake) potentially leading to misreporting. Lastly, $IDAA_{1C}$ score does not take into account hypoglycemia and glucose variability which are both important characteristics of PR (168).

Mechanisms underlying PR remain only partly understood. Restoration of metabolic balance following initiation of insulinotherapy (e.g., absence of ketones, reduction of lipids, normoglycemic state) may lead to a **partial recovery of immunotolerance** (e.g., increased T-cell function, upregulation of PDL-1, increase of $CD25^{+}$ 127^{high} memory cells), an **improvement of the microenvironment of β cells** (less inflammation, gluco- and lipotoxicity) and an **increase of insulin sensitivity**. These phenomena may consequently **reduce β-cell stress** (i.e., oxidative and RE stress, with reduction of apoptosis), and trigger both **a partial recovery of their function** (e.g., secretion) and their **relative escape from the immune system** (i.e., decrease of antigen presentation and HLA-I). Multiple findings supported these immune-metabolic changes with remitters showing decreased proinsulin/C-peptide ratios (181), increased residual secretion (168,182), increased insulin sensitivity (179), restoration of PD-1/PDL-1 expression (183) and specific immune and inflammatory signatures (184–187) as compared to non-remitters. While mainly described in mouse models of T1D, a small increase of β-cell mass by **transdifferentiation** of α-cells was proposed as an additional mechanism of PR (117,188–190).

Though limited in time, both occurrence and duration of PR showed a residual effect on short and long-term glucose homeostasis. From a short-term perspective, we recently showed that remitters had better short-term glucose homeostasis at 6 months after PR ends (i.e., $IDAA_{1C}$ and $GTAA_{1C}$ scores) as compared to non-remitters (**see 4.5**). Moreover, these differences increased proportionally to PR duration (i.e., long PR > short PR groups) (**see 4.5**). From a long-term perspective, patients that previously underwent PR showed a decrease in their insulinoresistance, HbA_{1C}, TDD, a higher residual β-cell secretion, a better lipid profile and a reduction of microvascular complications in the months to years after its end (146,173,191–194). While being clinically intuitive, these findings should be investigated in larger cohorts with a focus on PR length. Mechanisms underlying this residual effect may include both prolongated effect of immune-metabolic changes surrounding PR (see above) and "metabolic" memory phenomenon. The latter supports the role of early hyperglycemia in the development of long-term complications (195,196).

Occurrence and duration of PR were associated to several demographic characteristics (i.e., ethnicity, gender, age at onset, number of IAb+, genetic) (172,197–202) and clinical parameters at diagnosis (i.e., HbA_{1C} levels, presence of DKA, insulinoresistance) (192,197,198,202). Interestingly, these factors differed between pediatric and adult populations (199). As an example, a young African female (i.e., <5 years old at onset) with 3 IAb presenting with DKA has a very low likelihood of undergoing PR. Occurrence of PR may be also influenced by early postdiagnosis behaviors such as performing regular physical activity (200). Nonetheless, combination of

these elements lacks both sensitivity and specificity to predict occurrence of PR (180,203).

Identification of new reliable (predictive) biomarkers of glucose homeostasis evolution (e.g., β-cell function and PR occurrence) becomes key for the stratification of patients with new-onset T1D as:

1. Patients with significant residual β-cell function at T1D onset may better benefit from β-cell preservation therapies.
2. Prediction of both occurrence and temporality of PR may allow the identification of the best timing window to initiate prevention trials.
3. Better characterization of mechanisms underlying T1D pathogenies may provide additional clues for patient-tailored approaches. These strategies may either focus on one or more elements including decreasing immunity or inflammation, alleviating β-cell stress, preserving β-cell vascularization, or regenerating β cells.

- PR is an intervention-prone period for preservation of residual β-cell function.
- Gold-standard definition of PR relies on the $IDAA_{1C}$ score.
- PR demonstrated a positive residual effect on short- and long-term glucose homeostasis.
- Current biological and clinical markers of PR lack both sensitivity and specificity in its definition and prediction.
- Identification of new markers of glucose homeostasis evolution is key to achieve patient-tailored therapies in T1D.

1.4 The multiple facets of glucose homeostasis evaluation after T1D onset.

1.4.1 Clinical parameters allow reliable monitoring of glucose homeostasis

Substitution by exogenous insulin is currently the only solution for patients with T1D to maintain good metabolic control and prevent the development of long-term complications. Indeed, studies showed that maintaining good glycemic control (i.e., HbA_{1C}<7%) from clinical diagnosis prevented the development of long-term microvascular complications (204–207).

As mentioned in ISPAD guidelines (21), regular medical follow-up (i.e., quarterly in Belgium) and day-to-day management of glucose homeostasis help to achieve these glycemic targets. While capillary blood glucose monitoring (SMBG) improved the self-management of diabetes a few decades ago (208), daily finger pricks (usually >5 per day in patients with T1D) are associated with increased psychological burden and complications (209). In 2014, the reimbursement of CGM in Belgium reduced the invasiveness and improved the accuracy of glucose homeostasis evaluation. Indeed, studies showed that introduction of CGM allowed:

1. Improvement of glucose homeostasis: sustained reduction of HbA_{1C} (210), better management of hypoglycemia (especially in young children), and evaluation of glucose variability (211–214).
2. Minimal-invasive calculation of $eHbA_{1C}$ (estimated HbA_{1C}). Nonetheless, discrepancies (up to 1% in some patients) between blood-measured HbA_{1C} and $eHbA_{1C}$ may be observed due to personal glycation factors or hemoglobin turnover (215,216).

While eHbA$_{1C}$ is poorly invasive, current clinical standards rely on blood-measured HbA$_{1C}$. Currently, glycemic sensor companies are developing new algorithms that integrate these personal factors to improve eHbA$_{1C}$ accuracy.

3. Remote monitoring of glucose homeostasis (i.e., telemedicine). The latter promptly participated in the stability of glucose-control levels during the COVID-19 pandemia (217,218).

Altogether, these observations support CGM devices as both precise and minimal-invasive tools to evaluate glucose homeostasis.

1.4.2 Discrepancies exist between residual β-cell secretion and clinical parameters of glucose homeostasis

While clinical parameters (e.g., HbA$_{1C}$, CGM metrics) prevail in routine follow-up, stimulated residual β-cell secretion is the primary outcome of most prevention studies. Nonetheless, mixed-meal tolerance (MMTT) or glucagon-stimulated test (GST) showed major limitations as C-peptide levels only weakly-to-moderately correlated with clinical parameters of glucose homeostasis (219–221). These results support the divergence between residual β-cell secretion and function and challenge residual C-peptide as a reliable marker of glucose homeostasis (168,222). Reasons underlying this imperfect correlation may include poor integration of insulin sensitivity and β-cell secretion threshold by stimulated C-peptide. Conversely, both CGM metrics and clinical parameters demonstrated strong correlations with each other. These results altogether support the role of CGM metrics as reliable biomarkers of glucose homeostasis and potential tools to monitor the response to prevention therapies (168). Strengthening this idea, CGM

recently demonstrated increased sensitivity and specificity for classifying patients within the early stages of the disease as compared to the current gold-standard method (i.e., oral glucose tolerance test) (223). Also, differences in plasma glucose values were observed in children developing IAb, before seroconversion (224).

1.5 Introduction and further steps towards the prevention of type 1 diabetes

As defined by World Health Organization, prevention of a disease corresponds to minimizing the burden of a condition and/or associated risk factors by delivering the best intervention (e.g., drugs) at the right moment (e.g., disease stages) to the good patient (e.g., endotypes). Intervention may either prevent the development of the disease itself (i.e., primary), slow down its progression (i.e., secondary), or prevent disease-related complications (i.e., tertiary).

1.5.1 From primary to tertiary prevention of T1D: many hurdles but encouraging results

Treatment by exogenous insulin remains unsatisfactory for patients with T1D (e.g., decreased QoL, development of complications). Preventing the development of islet autoimmunity or lengthening the "insulin-free" period is a mainstream healthcare goal. Current strategies in T1D focus on:

1. Identifying newborns that are at risk to develop AID (i.e. primary prevention; before the onset of autoimmunity)
2. Identifying IAb-positive children that are at risk of progression to overt T1D (i.e. secondary prevention; stage 1 and stage 2)

3. Preserving residual β-cell function after clinical onset (i.e., tertiary prevention; stage 3)

Identification of at-risk or pre-symptomatic individuals is challenging as more than 90% of patients with T1D do not have any affected relatives (16). On the one hand, GRSs allowed the identification of newborns with high risk to develop β-cell autoimmunity in the general population (e.g., Global Platform for Prevention of Autoimmune Diabetes and The Environmental Determinants of Diabetes in the Young consortiums) (225–227). On the other hand, measurement of IAb during childhood may allow the identification of pre-symptomatic patients (228). Interestingly, combination of these markers improved both sensibility and specificity to predict the risk of developing T1D (229). Recent studies showed that T1D screening programs improved both patient's coping at T1D onset (e.g., smoother transition to insulin therapy, reduced psychological burden) (230,231) and peridiagnostic metabolic control (e.g., decrease of DKA episodes, higher residual C-peptide and better early glucose control) (231–233). Following these encouraging results, a group of experts proposed two distinct phases for early T1D screening (229):

1. Identification of multiple IAb+ children among the general population using multiple IAb screenings in early childhood (i.e., 3-5 years of age) and preadolescence (i.e., 11-13 years of age). The latter may allow the initiation of secondary prevention trials.

2. Screening of newborns in the general population (GRSs) followed by risk-driven IAb screening. The latter may allow the initiation of primary prevention trials.

Once identified, high-risk patients should be followed-up, educated on early signs of T1D and offered the possibility for interventional trials according to their disease stage. Interestingly, integration of GRSs in the newborn screening showed to be cost-effective in some pilot studies (69,234,235). Nonetheless, whether the latter should enter the routine population-wide screening remains debated as no disease-modifying drugs are, to date, accepted for T1D (69,236,237).

While some prevention therapies showed encouraging results, the majority failed to achieve their primary outcome independently of T1D stage (69,238,239). This paragraph provides a small summary of non-antigen specific immunotherapies though antigen-specific interventions or strategies targeting the β cell also showed some promising results (240). Detailed information on prevention therapies can be found in recently published reviews (69,227,229,238,240). Trials either targeting early diet modifications or insulin tolerization shortly after birth (i.e., primary prevention trials) yielded poor-to-intermediate results in preventing the development of islet autoimmunity with conclusions from the largest study being awaited for 2025 (i.e., Primary Oral Insulin Trial study) (69,227). Looking at secondary prevention interventions, administration of teplizumab (i.e., anti-CD3) in stage 2 patients provided a breakthrough in T1D field as it showed to delay disease progression (i.e., increase of insulin-

free period) and reduce the number of patients with new-onset T1D (241). These results led U.S. Food and Drug Agency to approve, for the first time, a T1D drug candidate as a delaying treatment in stage 2 patients in November 2022. Finally, some tertiary prevention trials delayed β-cell secretion decline after clinical onset (e.g., anti-CD3, anti-CD20, ATG, anti-TNF-α, abatacept, imatinibn alefacept (69,242)). Interestingly, careful analysis of the latter showed heterogeneity in the therapeutic response among participants with the identification of "responders" (i.e., patients showing good and extended clinical response) (243). These patients were characterized by trial-specific phenotypic and biological parameters, supporting the importance of patient stratification (138,244).

1.5.2 T1D prevention landscape, towards new goals and perspectives

Finding the Achille's heel of T1D relies on the integration of both immune and β-cell susceptibility, a better understanding of disease heterogeneity, and the identification of reliable biomarkers to stratify patients and evaluate therapeutic response (138,169,245). In that regard, future prevention therapies will need to focus on both blocking the immune reaction (i.e., cytokines or immune cell-directed interventions [antigen or non-antigen-specific]) and enhancing the residual β-cell function (i.e., increase of β-cell mass, relief of β-cell stress). As both processes likely happen at the same time, combined therapies may yield better results (138,244–247). Characterization of physiopathologies under T1D entity is also key to stratify patients and achieve successful patient-tailored therapy (138,247). Identification of early biomarkers that reliably predict and/or reflect clinically relevant parameters or outcomes (e.g., precise evaluation of

glucose homeostasis or diabetes-associated complications) becomes thus mandatory. In this context, CGM metrics may become new clinically reliable markers of residual β-cell function that allowed on the one hand, a minimal-invasive real-time evaluation of glucose homeostasis (including glucose variability), and on the other hand showed strong correlations with current routine markers of diabetes. As T1D prevention may be initiated at different stages of the disease, outcomes must be individualized and reliably witness either the apparition of islet autoimmunity or the evolution of β-cell function. Finally, the integration of minimal-invasive methods to evaluate the evolution of β-cell function during trials (e.g., CGM metrics rather than MMTT to monitor therapeutic response) may improve the recruitment of patients and accelerate the discovery of new preventative interventions (**see 1.5.3**).

1.5.3 Integration of biomarkers to picture and predict the disease evolution among patients with T1D

As preclinical models of T1D are questionable (238) and pancreatic samples are not available in the clinical routine (e.g., pancreas tissue or pancreatic fluid), researchers mostly rely on peripheral blood samples, non-invasive imaging of the pancreas, or CGM data to identify markers of immune reaction or β-cell (dys)function.

Circulating IAbs are the most common biomarkers of T1D. Nonetheless, these showed poor specificity in predicting the progression to T1D, the evolution of residual β-cell function, the stratification of patients, or the response to intervention therapies. As discussed in 1.3, an array of markers recently improved the understanding of disease heterogeneity and

the stratification of patients with new-onset T1D (152,159,164,184,248–253). Cross-sectional analysis of multi-omics data recently identified distinct biomarker signatures among high-risk and new-onset patients with T1D picturing specific disease evolution (170,254). However, to date, only IAbs and circulating C-peptide levels are used in the clinical routine to stratify these patients.

Additional minimal-invasive markers have raised interest in T1D research. Indeed, CGM metrics strongly correlated with current clinical parameters of glucose homeostasis and witnessed residual β-cell function (168). Furthermore, combination of specific CGM metrics yielded the identification of glucotypes among both pediatric patients with newly onset (168) and long-term T1D (255) that may foster patient-specific interventions. Multiparametric magnetic resonance imaging (MRI) may be another emerging tool that allows the longitudinal *in-vivo* evaluation of T1D pancreas from 5 years old (**see 4.3**). While current results of MRI studies did not provide substantial breakthroughs in the field of T1D, results from the MAP-T1D consortium are deeply awaited (256). Finally, PET-scan and radiolabeling recently demonstrated moderate correlation between residual β-cell mass and glucose homeostasis (i.e., glucose variability and TIR) (257).

2 Objectives of the work

Treatment of T1D relies on multiple injections of exogenous insulin as no curative therapy exists. Current inability to preserve residual β-cell function and/or halt the autoimmune process is grounded by multiple factors including a poor understanding of T1D physiopathology (i.e. autoimmunity, β-cell susceptibility, role of the exocrine pancreas), clinical heterogeneity (i.e. clinical presentation, residual β-cell function evolution [e.g. occurrence of partial remission], histology) and lack of reliable markers of the evolution of β-cell function evolution.

In this context, we aimed at better characterizing the first year following T1D diagnosis and identifying new (predictive) biomarkers of glucose homeostasis and PR. We initiated the multicentric DIAbetes TAGging (DIATAG) study that integrates a multilevel translational approach (e.g., plasmatic proteomic, genomics, residual β-cell secretion tests, CGM, MRI) to stratify pediatric patients at clinical onset of T1D according to their glucose homeostasis short- and mid-term evolution (**see point 3**).

Firstly, we studied the evolution of residual pancreatic endocrine function shortly after the clinical onset of T1D. On the one hand, we investigated whether routine markers of diabetes and PR (e.g., $IDAA_{1C}$ score, TDD, HbA_{1C}) correlated with either residual β-cell secretion (i.e., primary objective of prevention studies) or various CGM metrics. As CGM metrics strongly correlated with routine markers of glucose homeostasis, we next evaluated whether their circadian rhythm deepened the characterization of

PR and provided new evidence on the heterogeneous evolution of glycemic balance during the first year after T1D (**see 4.1**).

Secondly, we investigated whether the specific glycemic pattern previously described by our team (i.e., post-hypoglycemic hyperglycemia [PHH]) (258) distinguished remitters from nonremitters, supported the clinical relevance of our newly identified glucotypes (168) and may foster patient-specific therapeutic interventions (GLUREDIA study) (**see 4.2**).

Thirdly, as T1D involves both endocrine and exocrine compartments, we performed a thorough *in vivo* analysis of the whole pancreas. A subset of DIATAG patients underwent multiparametric MRI to characterize the longitudinal evolution of pancreas structure during the first year of T1D (i.e., at diagnosis and +12 months). We also investigated whether these MRI measures correlated and/or predicted pancreatic endocrine and exocrine functions (**see 4.3**).

Fourthly, we aimed to identify new predictive biomarkers of residual β-cell function evolution to stratify patients at diagnosis for prevention trials. We screened the plasma proteome of patients with T1D at clinical onset and correlated protein abundances to PR occurrence at 3 months postdiagnosis. Targeted validation was next performed on raw plasma for identified candidates (**see 4.4**).

Fifthly, most tertiary prevention trials aim to preserve and extend PR. We thus investigated whether the occurrence and the intensity of PR showed a residual effect on short-term glucose homeostasis. We retrospectively studied routine parameters of diabetes (i.e., TDD, HbA_{1C},

$IDAA_{1C}$, $GTAA_{1C}$) in the year following the end of PR and compared these to patients that did not undergo PR (DIABHONEY) (**see 4.5**).

In this thesis, we integrated a wide range of both gold standard and novel tools to improve the characterization of T1D heterogeneity (e.g., PR, endotypes). Also, we sought to identify clinical-friendly markers that reliably witnessed and/or predicted the evolution of glucose homeostasis during the first year of T1D. We hope that our findings will provide additional clues for prevention studies to reliably evaluate therapeutic responses and decrease the need for recurrent invasive testing (e.g., MMTT).

3 Genotypic and phenotypic characterization of new-onset type 1 diabetes children, the DIATAG study

3.1 Rationale and aims of DIAbetes TAGging (DIATAG)

T1D remains one of the few autoimmune diseases without curative treatment. As previously discussed in **1.4**, most prevention trials that aim to preserve residual β-cell secretion after clinical onset globally failed to reach their primary outcome as T1D heterogeneity is poorly understood and current biomarkers lack clinical reliability to stratify patients. In this context, the DIATAG consortium was initiated to better characterize pediatric patients with new-onset T1D during the first year after disease onset, especially during the PR period.

The primary objectives were:

- To extensively characterize T1D and PR using a multilevel approach. We performed cross-sectional analysis of various parameters including commonly advocated measures (e.g., residual secretion tests, glucose homeostasis parameters [e.g., HbA_{1C}, TDD, $IDAA_{1C}$]) and new additional measures (e.g., exocrine function, multiparametric pancreas imaging, plasmatic proteomic or miRNAs, circadian analysis of CGM data). Data from plasmatic miRNA screening study were not included in this thesis.
- To identify predictive biomarkers of PR in easily collectible samples while remaining poorly invasive
- To create a biobank containing longitudinal samples of serum, plasma, peripheral blood mononuclear cells [PBMC], urine, saliva, and feces.

The secondary objective was:

- To establish new prediction models of short and midterm β-cell function evolution using a combination of previously identified markers.

This thesis will focus on specific aspects of DIATAG trial and include glucose variability, pancreatic MRI analysis, and plasma proteome profiling.

3.2 Description of the DIATAG consortium and cohort

3.2.1 The DIATAG consortium and collaborators

The DIATAG consortium was built following a global impulse of seven Belgian pediatric diabetes centers under the umbrella of Pr. Philippe Lysy. Pediatric diabetes centers included CHC MontLégia (Liège, Belgium), CHR de la Citadelle (Liège, Belgium), Cliniques universitaires Saint-Luc (CUSL, Brussels, Belgium), Grand Hôpital de Charleroi (Charleroi, Belgium), UZ Antwerpen (Antwerp, Belgium), UZBrussel (Brussels, Belgium) and CHU UCL Namur (Yvoir, Belgium).

A summary of members participating in the DIATAG working group can be found hereunder.

Lysy A. Philippe (Cliniques universitaires Saint-Luc) ; Pollé G. Olivier (Cliniques universitaires Saint-Luc) ; Delfosse Antoine (Cliniques universitaires Saint-Luc) ; Gallo Paola (Cliniques universitaires Saint-Luc) ; Barrea Thierry (Cliniques universitaires Saint-Luc) ; De Valensart Gaetan (Cliniques universitaires Saint-Luc) ; Brunelle Chloé (Centre hospitalier Wallonie-Picard [CHwaPI]) ; Docquir Joachim (Grand Hôpital de Charleroi [GHdC]) ; Louis Jacques (Grand Hôpital de Charleroi [GHdC]) ; Oberweis Nicolas (Grand Hôpital de Charleroi [GHdC]) ; Gies Inge (Universitair Ziekenhuis Brussel) ; Staels Willem (Universitair Ziekenhuis Brussel) ; Vanbesien Jesse (Universitair Ziekenhuis Brussel) ; Van den Brande Christel (Universitair Ziekenhuis Brussel) ; Den Brincker Marieke (Universitair Ziekenhuis Antwerpen) ; Van Eyde Mieke (Universitair Ziekenhuis Antwerpen) ; Seret Nicole (Clinique CHC Montlégiat); Chivu Olimpia (Clinique

CHC Montlégiat) ; Lambert Sophie (Clinique CHC Montlégiat) ; Courtois Audrey (Clinique CHC Montlégiat) ; Lebrethon, Marie-Christinne (CHU Liège) ; Parent Anne-Simone (CHU Liège) ; Sondag Catherine (CHU Liège) ; Beckers Dominique (Centre Hospitalier universitaire Mont-Godinne) ; Moureau Thierry (Centre Hospitalier universitaire Mont-Godinne) ; Boutsen Laure (Centre Hospitalier universitaire Mont-Godinne).

Furthermore, experts from different fields were gathered to support distinct parts of the project including:

- Sébastien Pyr dit Ruys and Didier Vertommen (PHOS, De Duve Institute, Brussels) – plasma proteome profiling.
- Nicolas Michoux, Frank Peeters, Gaetan Duchêne (IMAG, UCLouvain, Brussels) and Philippe Clapuyt (cliniques universitaires Saint-Luc, Brussels) – technical and statistical MRI support.
- Laurent Gatto and Manon Martin (CBIO, De Duve Institute, Brussels) – methodological, statistical, and bioinformatic support.
- Jonathan Bavay (SmartSkills, Brussels) – big data management and development of PHH algorithm.
- Damien Gruson (cliniques universitaires Saint-Luc, Brussels) – Trypsinogen, C-peptide and plasmatic glucose measurements.
- Etienne Marbaix (cliniques universitaires Saint-Luc, Brussels) – biobank.

3.2.2 Study design and management

DIATAG is designed as a prospective pediatric longitudinal, multi-centric and non-pharmacological trial in which participants are evaluated every three

months during the first year after T1D diagnosis (i.e., short and mid-term follow-up). Management of diabetes was performed according to ISPAD guidelines (259). DIATAG trial also includes a long-term follow-up corresponding to a yearly evaluation for a total of six years. Study-specific evaluations included residual β-cell secretion testing using GST (+3 and +12 months) and pancreatic MRI scans (around diagnosis and +12 months). Residual β-cell secretion measures included $CPEP_{BASAL}$ (fasten value), $CPEP_{STIM}$ (AUC during GST test), $CPEP_{EST}$ (where loge (CP_{EST} + 1) = 0.317 + 0.00956 × BMI − 0.000159 × duration of T1D [days] + 0.710 × Fasting C-peptide [nmol/L] − 0.0117 × Fasting plasma glucose [mmol/L] − 0.0186 × HbA_{1c} [%] − 0.0665 × insulin dose [U/kg/day]). Detailed flow diagram of DIATAG study is shown in **Fig. 5**.

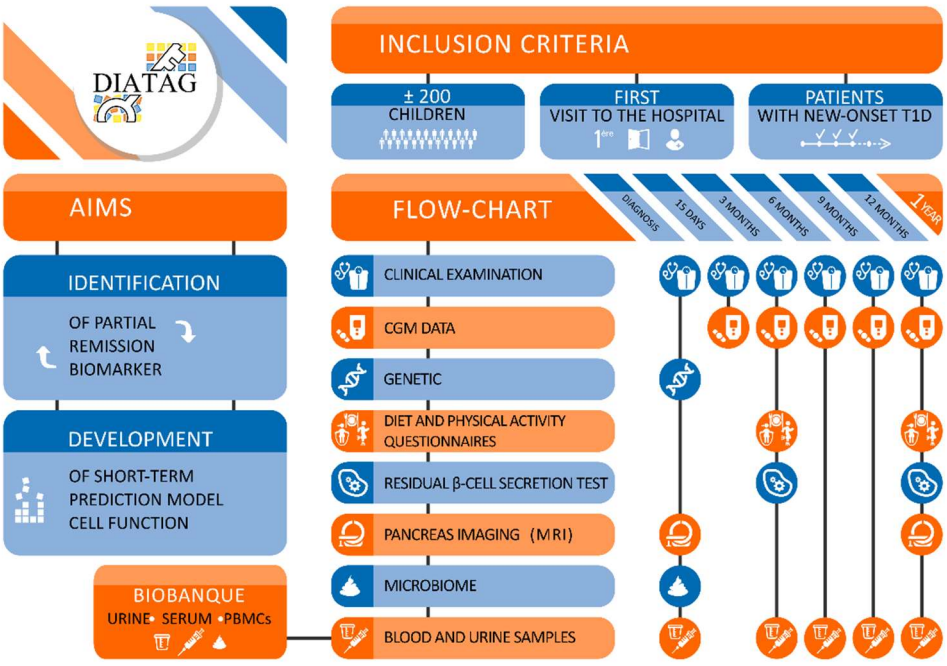

Figure 5: **Flow diagram of DIATAG study.**

As standardized preanalytical conditions are essential to reduce batch effect and improve data quality, most samples were treated on-site within 2-hours (i.e., urine, plasma, serum, feces, saliva) or 24-hours (i.e., PBMC isolation) after sampling using the same protocols across all laboratories. When PBMC isolation was not possible on-site (in all centers except UZ Antwerpen and CHR de la Citadelle), whole blood samples were retrieved at room temperature within 24 hours using laboratory shuttles and further treated at CUSL. All samples were centralized and stored at -80°C in CUSL biobank until further analysis. Also, C-peptide measurements were centralized in CUSL facilities.

The data management was performed using REDCap (Research Electronic Data Capture) platform. The trial was registered on www.clinicaltrial.gov (NCT04007809).

3.2.3 Inclusion and exclusion criteria

Patients included in the DIATAG study had a new diagnosis of T1D based on the ISPAD guidelines (69) and were aged between 6 months and 18 years old. Detailed inclusion and exclusion criteria are shown in **Table 4**.

Inclusion criteria	*Exclusion criteria*
Diabetes according to ISPAD criteria: 1. Polyuria, polydipsia, weight loss and sometimes ketoacidosis 2. Fasting blood glucose ≥126 mg/dL OR blood glucose ≥200 mg/dL at the 120th minute of an OGTT OR HbA1c ≥6.5% OR a patient with symptoms of hyperglycemia **and** hyperglycemic crisis with random blood glucose ≥200 mg/dL. 3. Presence of one or more anti-islet autoantibodies (anti-insulin, anti-IA2, anti-GAD65, anti-ZnT8) in the serum	Patient taking medications that may affect the insulin secretion and/or glucose homeostasis (i.e. corticosteroids, sulfonylurea, incretins, immunomodulatory drugs) before T1D diagnosis
Male or female	Presence of severe chronic medical conditions before the diagnosis of T1D (i.e. autoimmune diseases other than T1D, active cancer, kidney or liver insufficiency)
Age between 6 months and 18 years	Diabetes-onset before the age of 6 months
Written consent	Presence of comorbidities at inclusion (i.e. high blood pressure (>P95), proteinuria (> 0.15 g/L) or body mass index > +3 SD according to Cole et al.)
	Absence of islet autoantibodies

Table 4: Inclusion and exclusion criteria of DIATAG trial.

3.3 DIATAG patients demonstrate similar characteristics to previously described cohorts

The DIATAG trial was conducted from June 2019 to September 2021 and included 98 new-onset pediatric patients with T1D (48 from CUSL, 6 from UNamur, 10 from GHdC, 6 from CHC MontLegia, 11 from UZBrussel, 12 from CHU Liège and 5 from UZ Antwerpen). From these patients, 5 were lost of follow-up during the study and 2 had partial data. Globally, patients were equally distributed for gender and pubertal status (female [51.1 %] and prepuber [50.5%]). Participants had a mean ± SD age of 10.2 ± 3.8 years at type 1 diabetes onset and were principally under MDI regimen (86%). Incidence of DKA at clinical onset was 36%. The baseline characteristics of the cohort are described in **Table 5**. The flow diagram of patients' distribution across DIATAG subsidiary studies is presented in **Fig. 6**.

In our study, 62% of patients underwent PR ($IDAA_{1C}$ <9) at 3 months after diagnosis. PR prevalence was similar in other studies and peaked at 3 months post T1D diagnosis (260). Nonetheless, high variability could be observed with prevalence ranging between 20 and 80% (173,175,177,202,261). Interestingly, in our cohort, prepubertal children were less prone to undergo PR as compared to pubertal children (46% *vs* 69%, p<0.0001). Also, nonremitters showed an increased incidence of DKA at clinical onset as compared to remitters (49% *vs* 27%, p<0.0001) with prepubertal children significantly experiencing a higher rate of DKA at clinical onset (39% *vs* 31%, p=0.05). Accordingly, younger age and the presence of DKA at clinical onset were both previously associated with a reduction of PR (177,262–265). Finally, girls tended to experience less frequently PR than boys (44% *vs* 56%, p=0.08) with prepubertal girls having the lowest incidence of PR (36%). These results corroborated some studies that observed a more frequent onset of PR (173,177) in male patients though no differences were found in others (175,261–263,266).

Table 5: DIATAG population characteristics

	Global	*Remission*	*No remission*	*p-value**
Characteristic	***(N= 93)***	***(N=58)***	***(N= 35)***	
Distribution				
Age — years	10.2 ± 3.8	10.8 ± 3.5	9.3 ± 4.1	0.051†
Sex — Female no. (%)	47 (51)	25 (43)	22 (63)	0.092‡
Pubertal — no. (%)	46 (50)	32 (56)	14 (40)	0.20‡
BMI (Z-score)	-0.8 ± 1.3	-0.6 ± 1.4	-1.0 ± 1.3	0.13†
Baseline diabetes characteristics				
HbA_{1C} — %	12.2 ± 2.1	11.9 ± 2.2	12.7 ± 1.8	0.082†
Presence of ketoacidosis — no. (%)	32 (36)	15 (27)	18 (51)	0.043‡
Glycaemia — mg/dL	471 ± 181	461 ± 193	485 ± 161	0.51†
Insulin administration				
MDI — no. (%)	79 (86)	51 (88)	28 (82)	0.54‡
Fasting and stimulated C-peptide‖				
$CPEP_{BASAL}$ (pmol/mL) — n=78	0.3 ± 0.2	0.3 ± 0.2	0.2 ± 0.2	0.046¶
$CPEP_{STIM}$ (pmol/mL/min) — n=52	0.5 ± 0.3	0.5 ± 0.3	0.4 ± 0.3	0.021¶
$CPEP_{EST}$ (pmol/mL) § — n=78	0.6 ± 0.3	0.6 ± 0.2	0.5 ± 0.3	0.007†

Legend: Plus–minus values are means ± SD. Percentages may not total to 100 because of rounding. *p-value calculated between Remission and Non-remission group results were considered as significant when under 0.05. † Student t-test, ‡ Chi-square, ‖ Parameters evaluated at +3 months after diagnosis, ¶ Wilcoxon-test § calculated as described by Wentworth *et al* (267). Abbreviations: HbA1C = Glycated hemoglobin level, $IDAA_{1C}$ = insulin dose-adjusted A1C, $CPEP_{BASAL}$= fasten C-peptide, $CPEP_{STIM}$= stimulated C-peptide AUC after glucagon stimulation test, $CPEP_{EST}$ = calculated according to Wentworth *et al.* (267), MDI = Multiple Daily Injection, NA = not applicable.

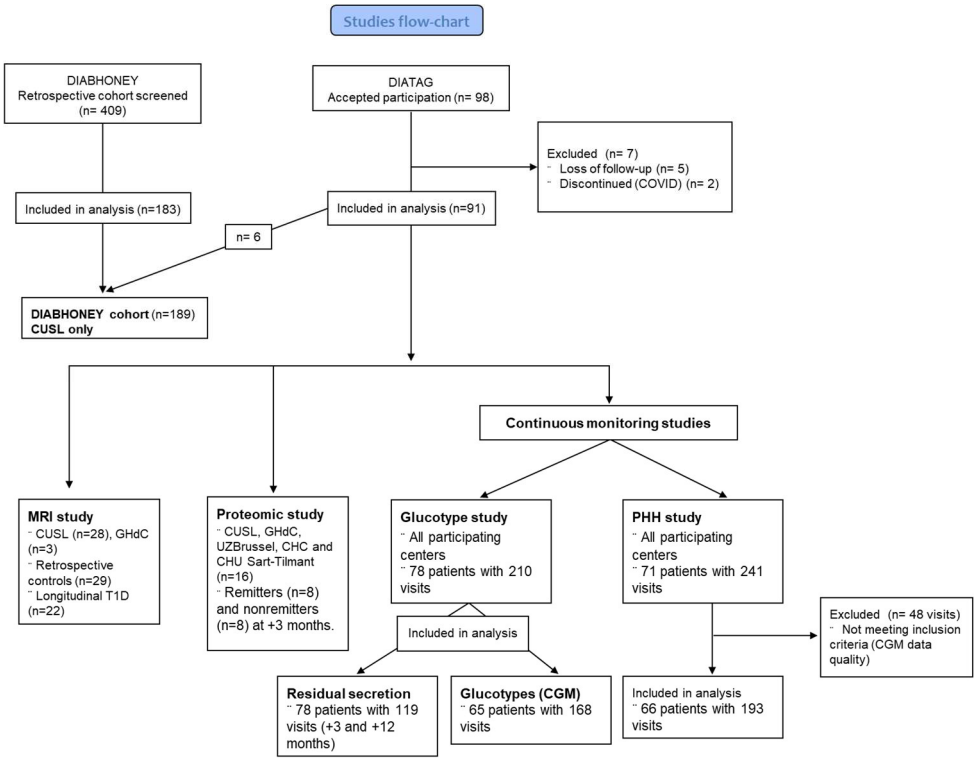

Figure 6: **Distribution of patients across studies included in the present thesis.** Abbreviations: CGM = continuous glucose monitoring, CHC = Centre hospitalier chrétient Mont-Légia, CUSL = Cliniques universitaires Saint-Luc, MRI = magnetic resonance imaging, GHdC = Grand Hôpital de Charleroi, PHH = Post-hypoglycemia hyperglycemia, T1D = type 1 diabetes

RESULTS

3.4 Paper I: Glycemic Variability Patterns Strongly Correlate with Partial Remission Status in Children With Newly Diagnosed Type 1 Diabetes

Published in Diabetes Care

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Short running title: Glycemic variability after diabetes onset

Key words: Type 1 diabetes, children, partial remission, glycemic variability, C-peptide, insulin dose-adjusted A1C, clustering.

3.4.1 Structured abstract

Objective: To evaluate whether indexes of glycemic variability may overcome residual β-cell secretion estimates in the longitudinal evaluation of partial remission in a cohort of pediatric new-onset type 1 diabetes patients.

Research Design and Methods: Values of residual β-cell secretion estimates, clinical parameters (e.g. HbA_{1C} or insulin daily dose) and continuous glucose monitoring (CGM) from 78 new-onset type 1 diabetes pediatric patients were longitudinally collected during one year and cross-sectionally compared. Circadian patterns of CGM metrics were characterized and correlated to remission status using adjusted mixed-effects model. Patients were clustered based on forty-six CGM metrics and clinical parameters, and compared using non-parametric ANOVA.

Results: Study participants had a mean (±SD) age of 10.4 (±3.6) years at diabetes onset and 65% underwent partial remission at +3 months. β-cell residual secretion estimates demonstrated weak-to-moderate correlations with clinical parameters and CGM metrics (r^2= 0.05-0.25, $p<0.05$). However, CGM metrics strongly correlated with clinical parameters (r^2 >0.52, $p<0.05$) and were sufficient to distinguish remitters from nonremitters. Also, CGM metrics from remitters displayed specific early morning circadian patterns characterized by increased glycemic stability across days (within 63-140 mg/dL range) and decreased rate of grade II hypoglycemia ($p<0.0001$), compared to nonremitters. Thorough CGM analysis allowed the identification of four novel glucotypes ($p<0.001$) that segregate patients into subgroups and mirror the evolution of remission after diabetes onset.

Conclusion: In our pediatric cohort, combination of CGM metrics and clinical parameters unraveled key clinical milestones of glucose homeostasis and remission status during the first year of type 1 diabetes.

3.4.2 Introduction

A major focus in modern medicine is recognizing disease heterogeneity and identifying measurable parameters for individualized health outcomes. Type 1 diabetes generally is characterized by ill-defined progressive immune-mediated β-cell destruction (1).

After a diagnosis of type 1 diabetes and initiation of insulin therapy, the evolution of the metabolic status of patients is marked by a dichotomy in their propensity to enter or not enter partial remission (PR), resulting from the preservation of residual β-cell function. Although variable in intensity and duration, PR is characterized by low levels of glycemic fluctuations and daily insulin needs that eventually result in the demise of β cells and a concomitant worsening of glycemic variability and glycated hemoglobin (HbA_{1C}) levels (2). For these reasons, the currently accepted definition of PR is provided by the calculation of an "insulin-dose adjusted HbA_{1C}" ($IDAA_{1C}$) score (3). Early and accurate identification of patients who will experience a significant PR period is key in developing secondary type 1 diabetes prevention strategies.

Most phase 3 interventional trials aimed at preserving β-cell mass after the diagnosis of type 1 diabetes have failed to meet the study's primary objectives, commonly defined as the persistence of C-peptide secretion above a certain threshold (e.g., peak C-peptide above 200 pmol/L) (4). Recent data from type 1 diabetes prevention trials demonstrated that only specific subgroups of patients might respond to the defined interventions based on this primary objective (5). Globally, the mitigated response of new-onset type 1 diabetes patients to a rather diverse portfolio of

pharmacological protocols supports the heterogeneity of type 1 diabetes and the need for patient stratification (6). It also challenges whether peak C-peptide estimation is the best metric for clinically significant (i.e., positively witnessing glucose homeostasis) residual β-cell function.

In patients with symptomatic type 1 diabetes, the evaluation of insulin secretion through standard oral tolerance testing poorly represents glucose homeostasis since it does not integrate key aspects of insulin sensitivity and since glucose responsiveness of β cells might only be observed in patients with high levels (i.e., >400 pM) of peak C-peptide (7,8). Since C-peptide assays lack the power to discriminate residual β-cell mass from β-cell function (9), new tools inferred from routine clinical parameters are needed that may both reflect the presence and predict the evolution of significant residual β-cell function, which qualifies PR.

HbA_{1C} variability is associated with a long-term risk of diabetes-related microvascular complications (10). With the generalized use of continuous glucose monitoring (CGM) systems, the intuitive clinical approach suggests that glucose variability parameters (also called CGM metrics) may strongly correlate with features of diabetes control related to β-cell function. This presumption is fueled by studies showing that CGM metrics (e.g., coefficient of variation, % of the time in hypoglycemia) refine the estimation of glucose control provided by HbA_{1C} measurement and may help to better stratify existing phenotypes among patients with type 1 diabetes (11,12).

The objectives of our subsidiary analysis of the DIATAG (DIAbetes TAGging) study are to evaluate whether CGM metrics correlate with clinical

parameters representing PR (e.g., HbA_{1C}, total insulin daily dose {TDD}, $IDAA_{1C}$) in pediatric patients with new-onset type 1 diabetes and how these CGM metrics may overcome residual β-cell secretion estimates from a longitudinal perspective, immediately after diagnosis. We thus also investigated how CGM metrics might help stratify patients according to the evolution of their level of glucose homeostasis during the first year of diabetes.

3.4.3 Research Design and Methods

Study design and participants

The DIATAG study was designed as a multicentric, prospective and nonpharmacological trial to identify biomarkers of PR in children and adolescents with new-onset type 1 diabetes. The study protocol was approved by the principal ethical committee (Comité d'Ethique Hospitalo-Facultaire of CUSL, 2018/04DEC/462) and local ethical committees of every participating institution. The parents and participants (>6 years old) gave their written informed consent prior to enrollment in the study. Patient enrollment is currently open. The trial is registered at www.clinicaltrial.gov (NCT04007809). Patients eligible for the study were aged 6 months to 18 years and were diagnosed with type 1 diabetes within the last 21 days. Type 1 diabetes was diagnosed according to the International Society for Pediatric and Adolescent Diabetes guidelines (13), which included the presence of at least one positive serum anti-islet autoantibody (anti-GAD, anti-ZnT8 transporter, anti-insulin, or anti-insulinoma-associated antigen-2). Exclusion criteria are detailed elsewhere (NCT04007809).

Study procedures

The baseline screening (i.e., Blood draws and urine was performed after an overnight fast between 5 and 21 days after diagnosis to allow metabolic stabilization. After the initial hospitalization, the outpatient clinical follow-up in diabetes care centers was organized throughout routine visits at +3, +6, +9 and +12 months, during which an array of data were collected (i.e., raw CGM, demographic and clinical parameters [i.e., TDD, HbA_{1C}, $IDAA_{1C}$] and insulin administration regimen [i.e., pump or multiple daily injections {MDI}]). All patients above four years old were recommended to wear CGM devices (Freestyle Libre®, Abbott; Dexcom®, Dexcom; Enlite™, Medtronic MiniMed). Data from the medical records of participants were gathered and registered inside the Research Electronic Data Capture (REDCap) system (14) provided by Vanderbilt University (Nashville, USA) and hosted at CUSL.

Glucagon Stimulation and β-cell Function Tests

A subset of participants (i.e., patients who completed the full study protocol) underwent a glucagon stimulation test (GST) at +3 months and +12 months to evaluate the insulin secretion capacities of β cells, as described elsewhere (15). In brief, after an overnight fast, patients were tested for capillary blood glucose. If pretest capillary glucose was between 70 and 250 mg/dL, 1 mg of glucagon (Glucagen®, Novonordisk) was injected intravenously. C-peptide and plasma glucose were measured at 1 minute preinjection and 2, 4, and 6 minutes postinjection. The C-peptide level was measured at the central laboratory of CUSL

(Brussels, Belgium) using a two-site chemiluminescence immunoassay (LiaisonXL®, Diasorin, France).

The response to GST was evaluated by calculating the area under the curve over a 6-minute interval as previously described (15), corresponding to $CPEP_{STIM}$ in this article. Peak C-peptide was determined as the maximal value of the latter during the GST test. $CPEP_{EST}$ (estimated C-peptide) was calculated as described elsewhere (16) using values of $CPEP_{BASAL}$ (i.e., fasting) and plasma glucose obtained before stimulation.

Remission status

Remission status was determined at each visit using the $IDAA_{1C}$ score as follows: HbA_{1C} (%) + (4 × insulin dose (U/kg body weight per 24 h), with a score below 9 defining the remission status (3). TDD was either reported by patients (i.e., MDI users) or calculated using the software for pump users.

Analysis of CGM data

CGM data were extracted at each outpatient clinical visit from a 30-day interval before measurement of HbA_{1C} (reference range 4–6% [20–42 mmol/mol]) (Tosoh G8, Tokyo, Japan). All CGM data were analyzed using R (R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.). Raw glycemic data were preprocessed using the R statistical package *cgmanalysis* (17). Data quality check (i.e., The exclusion of datasets if the time elapsed between CGM values was >45 minutes and the calculation of a panel of forty-six CGM metrics

(**Supplemental Table S1**) was performed using the *Iglu* package that provides functions for outputting relevant metrics for data collected from CGM (18,19). These were further classified into eight clusters (i.e., global control measures, hypoglycemia, time in range, hyperglycemia, within-day and between-day and total glucose variability, and combination scores). These were further classified into eight clusters (i.e., global control measures, hypoglycemia, time in range, hyperglycemia, glucose global, within- and between-day variability, and combination scores). Hypoglycemia and hyperglycemia were classified in grade 1 (i.e., 54-70 mg/dL [GIH_{54-70}], 180-250 mg/dL) or grade 2 (i.e., <54 mg/dL [$GIIH_{<54}$], >250 mg/dL) according to recommendations of the International Consensus on Use of Continuous Glucose Monitoring (20).The distribution of indices across global, within- and between-day variability was performed according to Rodbaar *et al* (21).

Statistical analysis

All statistical analyses were performed in R (R Core Team, 2021). The statistical significance level used for all analyses was 0.05. When appropriate and unless specified otherwise, data were log-transformed using $y=\log_e(value + 0.0001)$ if 0 values were included. Demographic and clinical data are reported as the mean ± SD for continuous variables and as numbers and proportions for categorical variables. Comparisons between groups were performed using Student's t test, chi-square test and linear regression or their nonparametric equivalent (Mann–Whitney U test, Fisher's exact test and Kruskal–Wallis test, respectively) as

appropriate. Multivariate regression was used to assess the impact of cofactors on peak C-peptide secretion. Cross-sectional comparisons between secretion, CGM and clinical parameters were performed using linear mixed models with R packages *lmer* (22) and *lmerTest* (23) to take into account multiple measurements from the same patient. Models included the methods as fixed effects and patient identity as a random intercept.

Four-week CGM data were analyzed either on a daily or hourly basis. Data densities were inspected in both datasets (i.e., daily or circadian analyses). Differences in circadian patterns of CGM metrics were investigated between remitters and nonremitters using a linear mixed model (22,23) that includes remission status (remitters/nonremitters) as a fixed effect and patient identity as a random intercept. Residuals were inspected for normality on Q-Q plots in which the distribution of the residuals' quantiles is compared to its theoretical normal one. This model was used to generate plots representing the amplitude of differences between both groups expressed in percentage of variation compared to the remitter group. P values were adjusted for multiple testing with the Benjamini & Hochberg FDR procedure (24).

Principal component analysis was conducted in R based on CGM data across all outpatient clinical visits (n=172). Prior to principal component analysis, glucose metrics were standardized and imputed by a regularized Expectation Minimization-PCA algorithm with the *missMDA* package (25). Next, unsupervised hierarchical clustering was performed

with the stats package (R Core Team, 2021) on standardized CGM data along the patients based on the Euclidean distance (i.e., CGM metrics). The number of clusters was determined based on a scree plot of the dendrogram height of the hierarchical clustering. As appropriate, comparisons between the groups were assessed by linear regression or its nonparametric equivalent (Kruskal–Wallis test).

3.4.4 Results

Study participant characteristics

The present subsidiary investigation of the DIATAG consortium study included 207 visits and 80 GSTs from 78 patients. All data were longitudinally collected during the first year after type 1 diabetes onset. Patients were mostly males (52%) and had a mean ± SD age of 10.4 ± 3.6 years at type 1 diabetes onset. The great majority of the cohort was under MDI (83.5%), and the incidence of PR (i.e., $IDAA_{1C}$ <9) at +3 months was 65%. The baseline characteristics of the cohort are provided in **Table 1**. $CPEP_{BASAL}$ was measured for 78 patients at +3 and +12 months (*n*=119), from which C-peptide was detectable (i.e., >0.01 pmol/mL) in all samples at +3 months and all but two samples at +12 months. On average, 1 GST was analyzed per patient (0 GST in 27 patients, 1 GST in 22 patients, 2 GST in 29 patients). None of the patients with undetectable fasting C-peptide had detectable C-peptide levels after stimulation.

Table 1: Characteristics of participants included in analysis

	Global	*Remission*	*No remission*	*p-value**
Characteristic	***(N= 78)***	***(N=51)***	***(N= 27)***	
Distribution				
Age — years	10.4 ± 3.6	10.9 ± 3.4	9.3 ± 0.4	0.09†
Sex — Male no. (%)	41 (52)	30 (59)	10 (37)	0.12‡
Pubertal — no. (%)	40 (51)	29 (57)	11 (40.7)	0.24‡
BMI (Z-score)	-0.7 ± 1.5	-0.6 ± 1.5	-1 ± 1.3	0.32†
Baseline diabetes characteristics				
HbA_{1C} — % [mmol/mol]	12.3[111] ± 2.1[23]	12.1[109] ± 2.2[24]	12.8[116] ± 1.8[19]	0.13†
Presence of ketoacidosis — no. (%)	26 (33)	13(26)	13(48)	0.04‡
Glycaemia — mg/dL	475 ± 190	462 ± 197	508 ± 193	0.31†
Weight loss — %	9.9 ± 15.47	6.9 ± 17.8	15.7 ± 6.8	0.003†
Insulin administration				
MDI — no. (%)	66 (83.5)	45(88)	20(77)	0.31‡
Insulin Pump — no. (%)	9 (11.4)	4(8)	5(19)	0.32‡
Unknown — no. (%)	3(5)	2(4)	1(4)	NA
Glycemic control‖ (n=78)				
HbA_{1C} — % [mmol/mol]	6.2[44] ± 0.7[8]	6[42] ± 0.5[6]	6.7[50] ± 0.8[8]	2.5e-20†
Insulin doses — IU/kg/day	0.6 ± 0.3	0.5 ± 0.2	0.8 ± 0.3	6.1e-20†
$IDAA_{1C}$	8.5 ± 1.3	7.8 ± 0.8	9.9 ± 1.1	3.4e-38†
Fasting and stimulated C-peptide‖				
$CPEP_{BASAL}$ (pmol/mL) — n=73	0.3 ± 0.2	0.3 ± 0.2	0.2 ± 0.2	0.01¶
Peak stimulated (pmol/mL) — n=52	0.7 ± 0.4	0.6 ± 0.4	0.4 ± 0.3	0.01¶
$CPEP_{STIM}$ (pmol/mL/min) — n=52	0.5 ± 0.3	0.5 ± 0.3	0.4 ± 0.3	0.02¶
$CPEP_{EST}$ (pmol/mL)§ — n=73	0.6 ± 0.3	0.6 ± 0.2	0.4 ± 0.2	2.6e-06†

Legend: Plus–minus values are means ±SD. Percentages may not total to 100 because of rounding. *p-value calculated between remitters and nonremitters results were considered as significant when under 0.05. † Student t-test, ‡ Chi-square, ‖ Parameters evaluated at +3 months after diagnosis, ¶ Wilcoxon-test § calculated as described by Wentworth *et al* (16). Abbreviations: HbA1C = Glycated hemoglobin level, $IDAA_{1C}$ = insulin dose-adjusted A1C, MDI = Multiple Daily Injection, NA = not applicable.

Residual C-peptide secretion estimates strongly correlate with each other but only weakly with clinical parameters.

In our DIATAG cohort, we wanted to determine whether C-peptide measured on a single fasted blood test (i.e., basal C-peptide [$CPEP_{BASAL}$] or model-based stimulated C-peptide [$CPEP_{EST}$]) was as efficient as the globally advocated "gold standard" stimulated C-peptide ($CPEP_{STIM}$) for evaluating residual endogenous secretion in children during the first year of type 1 diabetes. The influences of various cofactors on C-peptide measures were investigated using multivariate regression and revealed that peak C-peptide depended on sex and age ($p<0.05$) but not on basal glycemia, BMI, or time of measurement ($p>0.05$). However, in our DIATAG study, we decided not to adjust secretion data for sex and age for the following reasons: first, C-peptide *vs.* sex and age correlations may be influenced by the disease pathogenesis itself more than by the specifically chosen parameters (26), and second, the inclusion of sex and age parameters in a recent model was shown to worsen its power to predict stimulated C-peptide (16).

All three methods ($CPEP_{BASAL}$, $CPEP_{EST}$ and $CPEP_{STIM}$) were very strongly correlated with each other (i.e., $R>0.84$, $p<0.001$), underlining the concordance of stimulated *vs.* fasted C-peptide measures. $CPEP_{EST}$ was available for most patients, so we used the latter as our standard measure of β-cell secretion for cross-sectional analysis of the whole study cohort.

We next investigated whether these residual β-cell secretion estimates correlated with clinical parameters (i.e., HbA_{1C}, TDD, $IDAA_{1C}$) and found only weak-to-moderate correlations between the latter (r^2 between 0.05 and 0.25) (**Fig. 1A-C**). Notably, only $CPEP_{EST}$ correlated with all clinical

parameters while exhibiting the strongest correlation with $IDAA_{1C}$ (r^2=0.25). These results did not differ when considering the remission status (*data not shown*).

CGM-derived metrics strongly correlate with clinical parameters but only weakly with endogenous insulin secretion.

Our next approach was to evaluate whether the time spent within different glycemic ranges and the coefficient of variation (CV) might better reflect clinical parameters than β-cell secretion estimates (**Fig. 1D-F**)**.** We analyzed 500000 interstitial glucose values corresponding to a mean of 3450 measures per patient. The percentage of time spent between 70 and 180 mg/dL (time in range, $TIR_{70\text{-}180}$), 63 and 140 mg/dL (time in target, $TIT_{63\text{-}140}$) and above 180 mg/dL ($TAR_{>180}$) demonstrated the strongest correlations with HbA_{1C} levels (r^2=0.52, r^2=0.6, r^2=0.67 p<0.0001) and $IDAA_{1C}$ (r^2=0.53, r^2=0.53, r^2=0.54, p<0.0001) while showing the weakest correlations with TDD (r^2=0.16, r^2=0.1, r^2=0.09, p<0.0001). In contrast, CV and time spent below 70 mg/dL ($TBR_{<70}$) showed a weaker or no correlation with clinical parameters (**Fig. 1D-F**).

Finally, we investigated how the CGM metrics reflected residual β-cell secretion estimates. As shown in **Fig. 1G-I**, $TAR_{>180}$ and $TIR_{70\text{-}180}$ demonstrated the highest correlations with $CPEP_{EST}$ (r^2=0.13, r^2=0.22; p<0.01), while CV was equivalent in its correlations with $CPEP_{EST}$ and $CPEP_{STIM}$ (r^2 =0.17; p<0.01). Interestingly, $TBR_{<70}$ did not correlate with β-cell residual secretion estimates (p=0.77) while nearly reaching significance for $GIIH_{<54}$ (p=0.06, *data not shown*). Altogether, these data suggest that residual β-cell secretion only moderately reflects glucose homeostasis levels

when evaluated using either clinical parameters (HbA_{1C}, $IDAA_{1C}$) or CGM metrics, especially hypoglycemia.

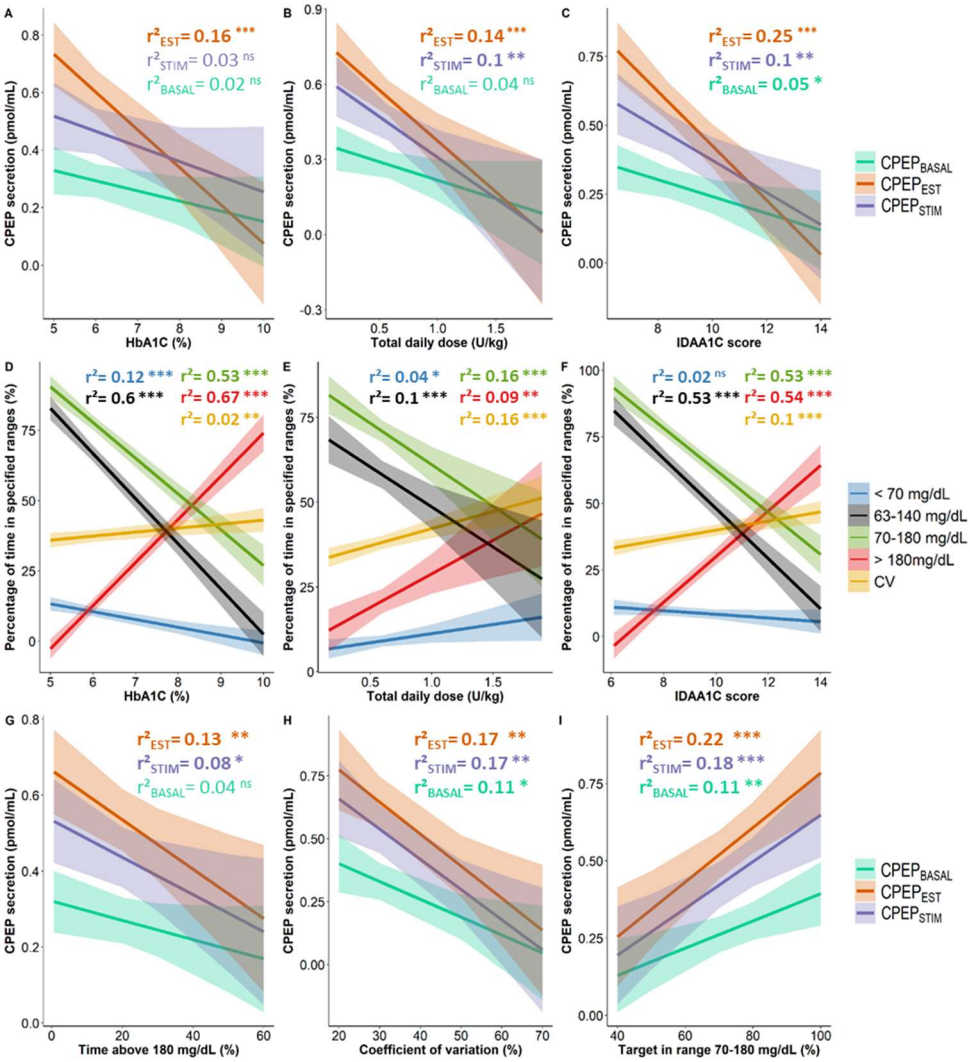

Figure 1: Relations between β-cell residual secretion, routine clinical parameters of glycemic control and CGM metrics during the first year of type 1 diabetes. Residual β-cell secretion was evaluated at +3 and +12 months after diagnosis. Routine clinical parameters and CGM metrics were obtained at +3, +6, +9, and +12 months after diagnosis. Correlation analyses were performed on all data. Panels A-C and G-I represent linear regression with 95% CI bands (shaded zone) between endogenous residual insulin secretion (i.e., $CPEP_{EST}$, $CPEP_{BASAL}$, $CPEP_{STIM}$) and HbA_{1C}

(A), daily insulin dose (B), $IDAA_{1C}$ score (C), time above 180 mg/dL (D), CV (E) and time between 70-180 mg/dL (F). Panels D-F represent linear regression with 95% CI bands (shaded zone) between CGM metrics (i.e., glycemia <70 mg/dL, between 63 and 140 mg/dL, between 70 and 180 mg/dL, and >180 mg/dL and CV) and HbA_{1C} (G), insulin daily dose (H) and $IDAA_{1C}$ score (I). Regression coefficients (r^2) are shown according to the secretion method (A-C, G-I) and CGM metrics (F-H). Abbreviations: CGM = continuous glucose monitoring; CV = coefficient of variation; HbA_{1C}, hemoglobin A1C; $IDAA_{1C}$ = insulin dose-adjusted A_{1C}. The level of significance of the correlations is represented after the r^2 value as follows: nonsignificant (ns), $p<0.05$ (*), $p<0.01$ (**), $p<0.001$ (***). Significant regression coefficients are indicated in bold.

CGM variables are robust and sufficient parameters to distinguish remitters from nonremitters

CGM metrics showed strong correlations with clinical parameters and allowed deeper characterization of glucose homeostasis (i.e., hypoglycemia episodes and glucose variability). We investigated whether a comprehensive analysis of CGM values might distinguish remitters from nonremitters. CGM metrics were analyzed on an hourly basis and compared with the patient remission status as a preanalytic evaluation of CGM demonstrated that specific periods of the day exhibited amplified differences between both groups (**Fig. 2**).

We first analyzed the time spent within different glycemic ranges, including hypoglycemia (i.e., $TBR_{<70}$, $GIIH_{<54}$), time in range (i.e., TIT_{63-140}, TIR_{70-180}) and hyperglycemia ($TAR_{>180}$). As expected, remitters spent more time in time in range (TIR_{70-180} [34%], TIT_{63-140} [57%]) and less time in hyperglycemia ($TAR_{>180}$ [-61%]) during the whole day (**Supplemental Table S2**). Moreover, while differences between both remission groups regarding time in range (TIR_{70-180}) were the highest during the day, differences in TIT_{63-}

$_{140}$ and $TAR_{>180}$ peaked in the early morning (i.e., 4-7 AM). During this specific period, we observed in remitters a peak in low-to-normal glucose values ($TBR_{<70}$ and TIT_{63-140}) but not in frank hypoglycemia ($GIIH_{<54}$), suggesting that most $TBR_{<70}$ episodes were in the 63-70 mg/dL range (**Fig. 2**)**.** The score assessing the risk of hypo- and hyperglycemia (average daily risk range score) remained low and stable over the 24 h in remitters while peaking during the day in nonremitters (**Supplemental Figure 1A**).

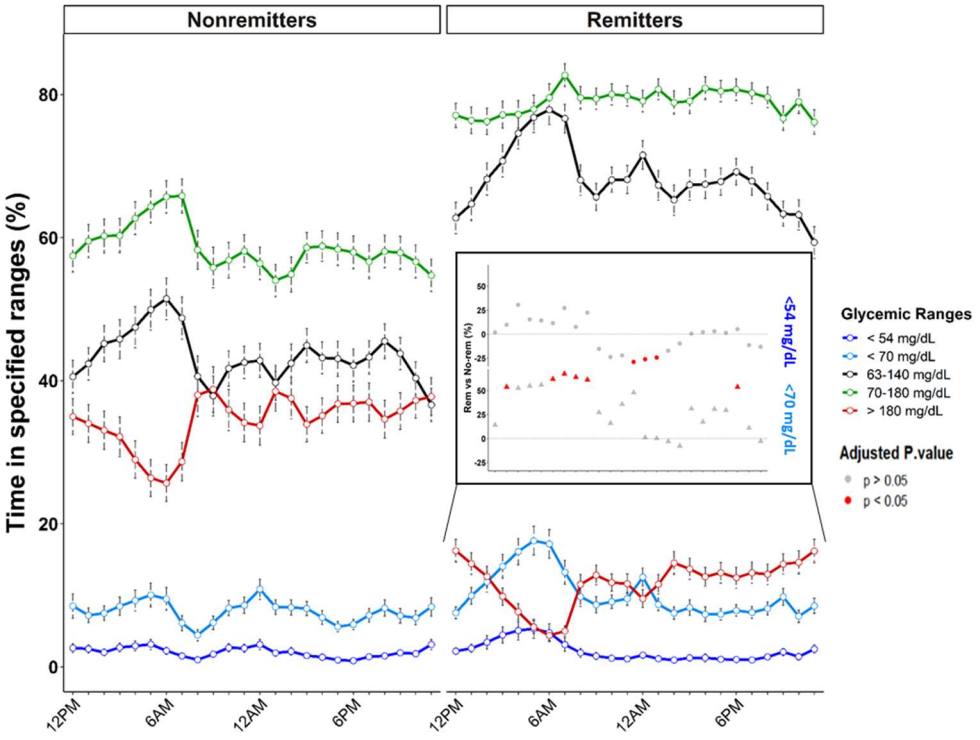

Figure 2: Daily patterns of time spent in defined glycemic ranges (A) during the first year of type 1 diabetes regarding the remission status. Routine clinical parameters and CGM metrics were obtained at +3, +6, +9, and +12 months after diagnosis. Lines represent the mean percentage of time spent below 54 mg/dL (dark blue), below 70 mg/dL (light blue), between 63-140 mg/dL (black), between 70-180 mg/dL (green) and above 180 mg/dL (red). Error bars represent the standard errors. The inserted panel represents the daily variation in the amplitude of differences for values below 70 mg/dL (triangle) and <54 mg/dL (round) between

remission groups based on a linear mixed model (i.e., the percentage above 0% [dashed black line] defining higher values in remitters, percentage below 0% defining lower values in remitters). The significance level of the differences is represented by the color of the points as follows: red dots correspond to p value <0.05, and gray dots represent p value >0.5.

Corroborating the early-morning glycemic pattern mentioned above, remitters experienced decreased total, between-day (interquartile range) and within-day glycemic variability throughout the whole day (i.e., CV <36% at all time points (27)), with differences between both groups reaching -22% and -49% for total and between-daybetween-day variability, respectively, at 6 AM (**Supplemental Figure 1B-C**).

Deep characterization of CGM metrics defines different remission clusters.

To investigate the evolution of glucotypes during the first year of type 1 diabetes, we performed a principal component analysis based on a panel of forty-six daily CGM metrics (**Supplemental Table S1**). The horizontal axis (PC1) was principally driven by hyperglycemia, time in range, within-day variability and global diabetes control indices, and the vertical axis (PC2) was driven by hypoglycemia and total variability indices (e.g., CV) (**Supplemental Figure 2A-B**). While PC1 segregated the HbA_{1C} and allowed the distinction between extreme values of $IDAA_{1C}$ (below 7.5 and above 10) (**Supplemental Figure 2C-D**), there remained an overlap between both remission groups for intermediate values (**Supplemental Figure 2D**).

To better understand the overlap between remission groups and identify subgroups of patients with similar glucose profiles, we performed unsupervised hierarchical clustering on CGM metrics and clinical parameters (**Supplemental Figure S3**). We identified four clusters of patients who were distinctly segregated across the principal component analysis (**Fig. 3A**) and

showed distinctive glycemic patterns (**Figs. 3B-C**). Groups significantly differed from each other for all clinical parameters and CGM metrics ($p<0.05$) (**Supplemental Table S3**). Metrics of time in range (TIR_{70-180} and TIT_{63-140}) were the highest in Group 1 but were progressively dissociated (i.e., decrease in TIT_{63-140}) during the daytime in Group 2 and the whole day in Group 3; a net drop in these values occurred in Group 4. Episodes of hyperglycemia ($TAR_{>180}$ and hyperglycemia >250 mg/dL) first appeared during the day in Group 2, extended to nighttime in Group 3 and peaked across the entire day in Group 4 (**Fig. 3C**). Regarding hypoglycemia, we found that the mean incidence of $TBR_{<70}$ was equivalently high in Groups 1 and 3 (**Supplemental Table S3**). However, as shown in remitters, $TBR_{<70}$ specifically increased in the early morning in Group 1 while remaining stable during the whole day in Group 3 with a concomitant increase of $GIIH_{<54}$.

Completing these observations, glycemic variability (CV and interquartile range) was the lowest in Group 1, increased in Group 2 and peaked in Group 3 during nighttime and in Group 4 during daytime. Notably, CV remained below the threshold of 36% in the first two groups (**Fig. 3B and Supplemental Table S3**).

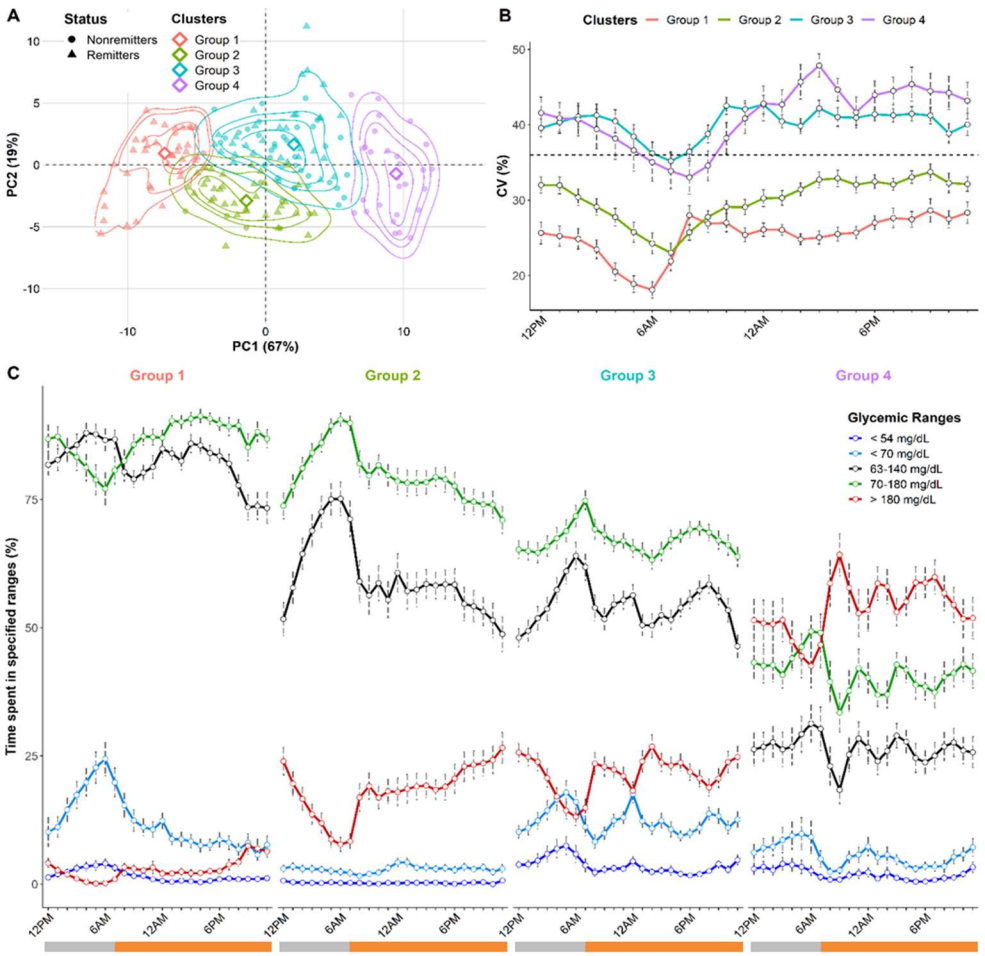

Figure 3: Illustration and characterization of glycemic clusters identified by unsupervised hierarchical clustering on CGM metrics and clinical data during the first year of diabetes. Routine clinical parameters and CGM metrics were obtained at +3, +6, +9, and +12 months after diagnosis. (A) Repartition of the clustering groups across the principal component analysis data. The empirical distributions of the patients across each group are represented by iso-probability contours of kernel densities at 25th, 50th, 75th, and 95th percentiles. The medoid of each group is represented by the diamond symbol. (B) Circadian evolution of CV (%) according to the clustering groups. The dashed line represents the threshold of 36%. (C) Daily patterns of time spent in defined glycemic ranges below 54 mg/dL (dark blue), below 70 mg/dL (light blue), between 63-140 mg/dL (black), between 70-180 mg/dL (green) and above 180 mg/dL (red). Error bars represent the standard errors. Gray horizontal bars represent nighttime, and orange horizontal bars represent daytime. Abbreviations: CGM = continuous glucose monitoring; CV = coefficient of variation.

3.4.5 Discussion and conclusions

Current methods for screening residual C-peptide secretion are rather invasive and poorly performed in reflecting the evolution of β-cell function and routine glucose parameters (28–31). A comprehensive composite evaluation of clinical parameters (i.e., TDD, HbA_{1C}, $IDAA_{1C}$) and CGM data are required to decipher glucose homeostasis evolution during the first year after type 1 diabetes onset and provide clues to achieve outcome-focused patient stratification (11).

Using cross-sectional measures of residual β-cell secretion, clinical parameters of glucose control and 4-week CGM, our study showed that clinical parameters (including $IDAA_{1C}$) showed a strong correlation with various CGM metrics yet a moderate correlation with β-cell secretion estimates. Using CGM data, we identified specific circadian patterns among remission groups for most CGM metrics (including $TIT_{63\text{-}140}$, $TBR_{<70}$ and between-day glucose variability), which peaked in their discriminative features in the early morning period. Finally, integrating CGM metrics and clinical parameters, we identified four clinically meaningful clusters that exhibit specific glucotypes and reflect the progressive loss of glucose homeostasis during the first year after type 1 diabetes onset.

There is controversy in using C-peptide as a forefront marker of residual β-cell function, as it fails to correlate with individual patient phenotypes (29,30). Accordingly, secretion estimates yielded a maximal correlation coefficient of 0.5 with routine parameters of glycemic control (i.e., HbA_{1C}, TDD, $IDAA_{1C}$), concurring results from previous studies (28–31). Our results support the study of Buckingam *et al.* (29), who demonstrated

that most new-onset patients with type 1 diabetes and $IDAA_{1C}$≥9 have detectable C-peptide secretion (>0.2 pmol/mL) in ranges not strictly parallel to HbA_{1C} levels. These observations illustrate the discrepant "C-peptide secretion vs. glucose homeostasis" discrepancy that can be attributed, at least partially, to individual insulin sensitivity and β-cell glucose responsiveness during the first postdiagnosis year (7–9,28,32).

When analyzing glycemic values, we hypothesized that CGM metrics ($TIR_{70\text{-}180}$ and $TAR_{>180}$) represent glucose homeostasis better than β-cell secretion testing (29,31). Using a proof-of-concept method, we found that routinely assessed CGM metrics allowed for a distinction between remitters and nonremitters. Daily glucose profile analysis identified the early morning as the most sensitive time to evaluate this distinction (**Fig. 2, Supplemental Fig. 1)**. Indeed, several metrics (e.g., $TBR_{<70}$, $TIT_{63\text{-}140}$ and interquartile range) were the most powerful for patient stratification, highlighting that a special focus on the early morning period might provide the highest yields in the search for analytic tools to segregate remitters from nonremitters.

Our analysis also fueled the characterization of hypoglycemia in remitters and the importance of the severity of hypoglycemic episodes as indirect markers of residual β-cell secretion. We demonstrated for the first time the predominance of early morning low-grade hypoglycemia in remitters compared to nonremitters (**Fig. 2**), suggesting that $TBR_{<70}$ in the 63-70 mg/dL range might be a clinically significant meaningful marker of β-cell function. This was partially suggested by previous studies showing frequent $TBR_{<70}$ in secretors (29), minimal increase in $TBR_{<70}$ in remitters (33), and a high proportion (>50%) of $TBR_{<70}$ in the 65 to 70 mg/dL range

during PR (33). However, we found for the first time that high-grade hypoglycemia ($GIIH_{<54}$) tended to negatively correlate with β-cell residual secretion, confirming that increased dependence on exogenous insulin fosters the occurrence of severe hypoglycemia in patients with new-onset type 1 diabetes.

To better understand the heterogeneity of type 1 diabetes evolution the first year after onset, we identified four clusters of glucotypes supporting the clinical impression that the emergence of glycemic dysregulations follows a continuum, first appearing during the daytime before progressing to the nighttime with a concomitant increase in glycemic variability, hyperglycemia and subsequent $GIIH_{<54}$ (**Fig. 3**). Additionally, our data also show that new-onset type 1 diabetes patients were distributed across all groups from +3 months (i.e., 38.2% in Group 1, 25.5% in Group 2, 29.1% in Group 3, 7% of patients in Group 4), highlighting that levels of glycemic dysregulations might be heterogeneous from the first months after diabetes onset. Altogether, these results provide new insights into understanding the patchiness of type 1 diabetes phenotypes (12) and challenge considerations of PR as a dichotomic event. In that regard, CGM metrics provide additional information to segregate patients, especially with intermediate $IDAA_{1C}$ values.

Our subsidiary analysis of the DIATAG cohort demonstrates several strengths. This is the first pediatric multicentric cross-sectional study that integrates CGM, clinical parameters and residual β-cell secretion data to uncover the characteristics of PR and identify new glucotypes during the first year of type 1 diabetes. Furthermore, cohort characteristics (e.g., the ratio

of MDI regimen, the same level of care) and user-friendly standardized methods to analyze CGM data support our classification's external validity and translational in clinical routine (18).

Our study was limited by cross-sectional analysis of all three parameters (i.e., clinic, secretion and CGM) that were only available for a subset of patients, as the study trial is still open. Nonetheless, the impact of these biases was limited. On the one hand, $CPEP_{EST}$ allowed reliable secretion measurement for the great majority of the patients (>95%). On the other hand, the first endpoint of CGM analysis was to depict an overview of type 1 diabetes glucotypes during the first year rather than evaluating the individual evolution of patients across the groups. The sensor manufacturer might also influence data. Notably, the impact is limited, as most of the data were obtained from Freestyle Libre® (i.e., >90%), and no sensor-specific pattern was observed within the principal component analysis.

Our study confirmed that β-cell secretion estimates, evaluated using either a single blood test or stimulation testing, were only weakly correlated with glucose homeostasis. CGM metrics (e.g., hyperglycemia and time in range) demonstrated a strong correlation with routine clinical parameters (i.e., HbA_{1C} and $IDAA_{1C}$ score) and demonstrated, for most of them, a specific circadian pattern that distinguished both remission groups, specifically in the early morning period. Moreover, we identified $TIT_{63\text{-}140}$, $GIIH_{<54}$ and between-day glucose variability as key parameters to distinguish remitters from nonremitters. Finally, we showed that using a combination of CGM metrics and clinical parameters allowed for identifying four categories of patient groups that experienced varying degrees of glucose homeostasis

during the first year of type 1 diabetes. We believe that integrating various CGM metrics as endpoints in residual β-cell function prevention trials might provide clinically relevant and precise clues to evaluate patient response to treatment protocols.

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Authors Contributions. O.P. contributed to the design, implementation and coordination of the study, collected and interpreted data, and wrote the first draft of the manuscript. A.D. contributed to the coordination of the study, collected and interpreted data, wrote the first draft and revised the manuscript. M.M. participated in the design and analysis of the data, and

wrote the first draft of the manuscript. L.G. participated in the design and supervised data analysis, and reviewed the first draft and revised the manuscript. L.J., I.G., M.D.B., M. L., T. M. contributed to the implementation and coordination of the study, collected the data, reviewed the first draft and revised the manuscript. P.L. conceived the research question and the study design, interpreted the data, wrote the first draft and revised the manuscript. P.L. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the analyses.

3.4.6 References

1. Van Belle TL, Coppieters KT, Von Herrath MG. Type 1 diabetes: Etiology, immunology, and therapeutic strategies. Physiol Rev. 2011;91(1):79–118.

2. Nielens N, Pollé O, Robert A, Lysy PA. Integration of Routine Parameters of Glycemic Variability in a Simple Screening Method for Partial Remission in Children with Type 1 Diabetes. J Diabetes Res. 2018;2018.

3. Mortensen HB, Hougaard P, Swift P, Hansen L, Holl RW, Hoey H, et al. New definition for the partial remission period in children and adolescents with type 1 diabetes. Diabetes Care. 2009;32(8):1384–90.

4. Palmer JP, Fleming GA, Greenbaum CJ, Herold KC, Jansa LD, Kolb H, et al. C-Peptide Is the Appropriate Outcome Measure for Type 1 Diabetes Clinical Trials to Preserve -Cell Function: Report of an ADA Workshop, 21-22 October 2001. Diabetes. 2004;53(October 2001):21–2.

5. Atkinson MA, Roep BO, Posgai A, Wheeler DCS, Peakman M. The challenge of modulating β-cell autoimmunity in type 1 diabetes. Lancet Diabetes Endocrinol. 2019;7(1):52–64.

6. Linsley PS, Greenbaum CJ, Nepom GT. Uncovering pathways to personalized therapies in type 1 diabetes. Diabetes. 2021 Apr 1;70(4):831–41.

7. Rickels MR, Evans-Molina C, Bahnson HT, Ylescupidez A, Nadeau KJ, Hao W, et al. High residual C-peptide likely contributes to glycemic control in type 1 diabetes. J Clin Invest. 2020;130(4):1850–62.

8. Yu MG, Keenan HA, Shah HS, Frodsham SG, Pober D, He Z, et al. Residual β cell function and monogenic variants in long-duration type 1 diabetes patients. J Clin Invest. 2019;129(8):3252–63.

9. Oram RA, Sims EK, Evans-Molina C. Beta cells in type 1 diabetes: mass and function; sleeping or dead? Diabetologia. 2019;62(4):567–77.

10. Gorst C, Kwok CS, Aslam S, Buchan I, Kontopantelis E, Myint PK, et al. Long-term glycemic variability and risk of adverse outcomes: A systematic review and meta-analysis. Diabetes Care. 2015;38(12):2354–69.

11. Kahkoska AR, Adair LA, Aiello AE, Burger KS, Buse JB, Crandell J, et al. Identification of clinically relevant dysglycemia phenotypes based on continuous glucose monitoring data from youth with type 1 diabetes and elevated hemoglobin A1c. Pediatr Diabetes. 2019;20(5):556–66.

12. Battaglia M, Ahmed S, Anderson MS, Atkinson MA, Becker D, Bingley PJ, et al. Introducing the endotype concept to address the challenge of disease heterogeneity in type 1 diabetes. Diabetes Care. 2020;43(1):5–12.

13. Mayer-Davis EJ, Kahkoska AR, Jefferies C, Dabelea D, Balde N, Gong CX, et al. ISPAD Clinical Practice Consensus Guidelines 2018: Definition, epidemiology, and classification of diabetes in children and adolescents. Pediatr Diabetes. 2018;19:7–19.

14. Harris PA, Taylor R, Minor BL, Elliott V, Fernandez M, O'Neal L, et al. The REDCap consortium: Building an international community of software platform partners. J Biomed Inform. 2019;95(May):103208.

15. Philippe MF, Benabadji S, Barbot-Trystram L, Vadrot D, Boitard C, Larger E. Pancreatic volume and endocrine and exocrine functions in patients with diabetes. Pancreas. 2011;40(3):359–63.

16. Wentworth JM, Bediaga NG, Giles LC, Ehlers M, Gitelman SE, Geyer S, et al. Beta cell function in type 1 diabetes determined from clinical and fasting biochemical variables. Diabetologia. 2019;62(1):33–40.

17. Vigers T, Chan CL, Snell-Bergeon J, Bjornstad P, Zeitler PS, Forlenza G, et al. cgmanalysis: An R package for descriptive analysis of continuous glucose monitor data. PLoS One. 2019 Oct 1;14(10):e0216851.

18. Broll S, Urbanek J, Buchanan D, Chun E, Muschelli J, Punjabi NM, et al. Interpreting blood GLUcose data with R package iglu. 2021;16(4):e0248560.

19. Fabris C, Patek SD, Breton MD. Are Risk Indices Derived from CGM Interchangeable with SMBG-Based Indices? Vol. 10, Journal of Diabetes Science and Technology. 2016. p. 50–9.

20. Danne T, Nimri R, Battelino T, Bergenstal RM, Close KL, DeVries JH, et al. International consensus on use of continuous glucose monitoring. Diabetes Care. 2017;40(12):1631–40.

21. Rodbard D. New and improved methods to characterize glycemic variability using continuous glucose monitoring. Diabetes Technol Ther. 2009;11(9):551–65.

22. Bates D, Mächler M, Bolker BM, Walker SC. Fitting linear mixed-effects models using lme4. J Stat Softw. 2015;67(1).

23. Kuznetsova A, Brockhoff PB, Christensen RHB. lmerTest Package: Tests in

Linear Mixed Effects Models . J Stat Softw. 2017;82(13).

24. Benjamini Y, Hochberg Y. Controlling the False Discovery Rate : A Practical and Powerful Approach to Multiple Testing Author (s): Yoav Benjamini and Yosef Hochberg Source : Journal of the Royal Statistical Society . Series B (Methodological), Vol . 57 , No . 1 (1995), Publi. J R Stat Soc. 1995;57(1):289–300.

25. Josse J, Husson F. missMDA: A package for handling missing values in multivariate data analysis. J Stat Softw. 2016;70(1).

26. Pecheur A, Barrea T, Vandooren V, Beauloye V, Robert A, Lysy PA. Characteristics and Determinants of Partial Remission in Children with Type 1 Diabetes Using the Insulin-Dose-Adjusted A1C Definition. J Diabetes Res. 2014;2014:1–7.

27. Monnier L, Colette C, Wojtusciszyn A, Dejager S, Renard E, Molinari N, et al. Toward defining the threshold between low and high glucose variability in diabetes. Diabetes Care. 2017;40(7):832–8.

28. Ruan Y, Willemsen RH, Wilinska ME, Tauschmann M, Dunger DB, Hovorka R. Mixed-meal tolerance test to assess residual beta-cell secretion: Beyond the area-under-curve of plasma C-peptide concentration. Pediatr Diabetes. 2019 May 1;20(3):282–5.

29. Buckingham B, Cheng P, Beck RW, Kollman C, Ruedy KJ, Weinzimer SA, et al. CGM-measured glucose values have a strong correlation with C-peptide, HbA1c and IDAAC, but do poorly in predicting C-peptide levels in the two years following onset of diabetes. Diabetologia. 2015;58(6):1167–74.

30. Hao W, Gitelman S, Di Meglio LA, Boulware D, Greenbaum CJ. Fall in C-peptide during first 4 years from diagnosis of type 1 diabetes: Variable relation to age, HbA1c, and insulin dose. Diabetes Care. 2016;39(10):1664–70.

31. Carr ALJ, Oram RA, Marren SM, McDonald TJ, Narendran P, Andrews RC. Measurement of Peak C-Peptide at Diagnosis Informs Glycemic Control but not Hypoglycemia in Adults with Type 1 Diabetes. J Endocr Soc. 2021;5(10):1–10.

32. Chmelova H, Cohrs CM, Chouinard JA, Petzold C, Kuhn M, Chen C, et al. Distinct roles of beta cell mass and function during type 1 diabetes onset and remission Running Title: Beta cell plasticity during diabetes remission. 2015;49(0):1–37.

33. Addala A, Zaharieva DP, Gu AJ, Prahalad P, Scheinker D, Buckingham B, et al. Clinically Serious Hypoglycemia Is Rare and Not Associated With Time-in-Range in Youth With New-Onset Type 1 Diabetes. J Clin Endocrinol Metab. 2021 Jul 15;

3.4.7 Online-only Supplemental data

Rationale to include supplemental data: We believe that including these supplemental data will allow a better understanding of the methodology and strengthen our findings in the characterization of glucose homeostasis during the first year after type 1 diabetes onset.

1. Global CGM metrics (Supplemental Table S1) distinguished remitters from nonremitters. Notably, there were no significant differences in grade I and II hypoglycemia daily values between the remission groups (Supplemental Table S2). *Time in target (TIT)* and *interquartile range (IQR)* are key characteristics of remitters. Additionally, remitters exhibited lower global and between-day glycemic variability during all the day, with the largest differences observed in the early morning (CV, IQR) (Supplemental Figure 1).
2. Principal component analysis (PCA) results strengthen the observation that partial remission is not a dichotomic event (i.e., $IDAA_{1C}$ < or >9) and that the $IDAA_{1C}$ score lacks sensitivity to distinguish remission groups for intermediate values (Supplemental Table S1, Supplemental Figure 1, and Supplemental Figure 2).
3. Hierarchical clustering on clinical parameters and CGM metrics allowed the identification of four distinct glucotypes during the first year after type 1 diabetes onset (Supplemental Table S3 and Supplemental Figure 3).

Supplemental Table S1

Table S1. Groups of the forty-six CGM metrics

Global control	*Euglycemia*	*Hypoglycemia*	*Hyperglycemia*	*Between-day variability*	*Within-day variability*	*Total variability*	*Combination score*
eA1C	GRADE eugly	GRADE hypo	GRADE hyper	IQR	MAGE	CV	COGI
AUC	% above 140 mg/dL	LBGI	Hyper Index	MODD	CONGA	ADRR	GRADE
M-value	% in 63-140 mg/dL	Hypo Index	% above 180 mg/dL	SD_b	SD_{ROC}	CV_{MEAN}	IGC
SD_T	% in 70-180 mg/dL	% below 54 mg/dL	% above 250 mg/dL	$SD_{b\ DM}$	MAG	CV_{SD}	J-index
1st quartile		% below 70 mg/dL	HBGI	SD_{DM}	SD_W		
Median					$SD_{hh:mm}$		
Mean					$SD_{ws\ h}$		
3rd quartile					GVP		
GRADE					MAD		
GMI							

Abbreviations: ADRR, average daily risk range; COGI, composite continuous glucose monitoring index; CONGA, continuous overall net glycemic action; CGM, continuous glucose monitoring; CV, coefficient of variation; eA1C, estimated hemoglobin A1c; GMI, glucose management indicator; GRADE, glycemic risk assessment diabetes equation; GVP, glycemic variability percentage; HBGI, high blood glucose index; IGC, index of glycemic control; IQR, interquartile range; LBGI, low blood glucose index; MAD, mean absolute difference; MAG, mean absolute glucose; MAGE, mean amplitude of glycemic excursions; MODD, mean of the daily differences; SD, standard deviation; SD_b, SD between days–within time points; $SD_{b\ DM}$, SD between days–within time points, after correction for changes in daily means; SD_{ROC}, SD of the rate of change; SD_{DM}, SD of daily means; SD_W, mean of daily SD; SD_T, total SD; $SD_{hh:mm}$, SD between time points; $SD_{w\ sh}$, SD within series.

Supplemental Table S2. Continuous glucose monitoring (CGM) metrics in remitters and nonremitters during the first year of type 1 diabetes (n= 65, 168 visits)

	Global	*Remission*	*No remission*	*p-value**
Characteristics	**(N= 168)**	**(N=95)**	**(N= 74)**	
Distribution				
Age — years	10.4 ± 4.7	10.7 ± 5.5	10.1 ± 3.3	0.4†
Sex — Male no. (%)	86 (50.9)	63 (66)	23 (34)	<0.0001‡
Pubertal — no. (%)	88 (52.1)	56 (59)	32 (43)	0.05‡
BMI (Z-score)	0.3 ± 1	0.2 ± 1	0.4 ± 1	0.2†
Glycemic control§				
HbA_{1C} — % [mmol/mol]	6.6[49] ± 1[11]	6[42] ± 0.5[6]	7.3[56] ± 1[11]	1.5e-17†
Insulin doses — IU/kg/day	0.6 ± 0.3	0.5 ± 0.1	0.8 ± 0.3	2.2e-19‖
$IDAA_{1C}$	9 ± 1.6	7.9 ± 0.7	10.5 ± 1.3	2.8e-30†
Fasting and stimulated C-peptide¶ (n=81)				
$CPEP_{BASAL}$ (pmol/mL) — n=81	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.3	0.06 ‖
Peak stimulated (pmol/mL) — n=61	0.5 ± 0.3	0.6 ± 0.4	0.4 ± 0.3	0.03 ‖
$CPEP_{GST}$ (pmol/mL/min) — n=61	0.43 ± 0.3	0.5 ± 0.3	0.33 ± 0.3	0.04 ‖
$CPEP_{EST}$# (pmol/mL) — n=81	0.5 ± 0.3	0.6 ± 0.3	0.4 ± 0.3	1.1e-05‖
Global control§				
Mean glucose (mg/dL)	139 ± 34	121 ± 19	161 ± 35	1e-14†
Hypoglycemia§				
Below 70 mg/dL — %	11 ± 8	12 ± 8	10 ± 6	0.06‖
Below 54 mg/dL — %	4 ± 4	4 ± 5	4 ± 3	0.28‖
LBGI	3.1 ± 1.7	3.4 ± 1.9	2.7 ± 1.2	0.002‖
Euglycemia§				
In range 63-140 mg/dL — %	58 ± 21	69 ± 16	44 ± 17	1e-17†
In range 70-180 mg/dL — %	70 ± 17	79 ± 11	59 ± 16	2.6e-15†
Hyperglycemia§				
Above 180 mg/dL — %	23 ± 18	14 ± 10	36 ± 19	1e-15‖
Above 250 mg/dL — %	9 ± 10	4 ± 3	15 ± 12	1.8e-11‖
HBGI	6.2 ± 4.9	3.6 ± 2	9.5 ± 5.6	1.6e-13‖
Total variability§				
Coefficient of variation — %	41 ± 9	38 ± 9	44 ± 8	2.1e-07‖
ADRR	37 ± 14	30 ± 10	46 ± 12	1.1e-16†
Within-day variability§				
SD_{ROC} (mg/dL)	2.5 ± 0.6	2.3 ± 0.4	2.8 ± 0.6	1.2e-08‖
CONGA	66 ± 26	53 ± 19	83 ± 24	2.3e-15†

Between-day variability[§]				
Interquartile range (mg/mL)	72 ± 31	56 ± 20	93 ± 30	2.4e-15[†]
MODD	51 ± 21	40 ± 14	65 ± 19	2.6e-16[†]
Combination score[§]				
COGI	60 ± 16	55 ± 13	65 ± 17	7.1e-06[†]

Legend: Plus-minus values are means ± SD. Percentages may not total 100 because of rounding. * p-value calculated between remitters and nonremitters, results were considered as significant when under 0.05. [†] student t-test; [‡] Chi-square; [§] parameters evaluated at +3, +6, +9, +12 months after diagnosis; [||] Wilcoxon-test; [¶] parameters evaluated at +3 and +12 months after diagnosis; [#] calculated as described in Wentworth *et al*. Abbreviations: ADRR, average daily risk range; COGI, composite continuous glucose monitoring index; CONGA, continuous overall net glycemic action; HBGI, high blood glucose index; HbA_{1C}, Hemoglobin A1C; LBGI, low blood glucose index; MDI, Multiple Daily Injection; MODD, mean of the daily differences; SD_{ROC}, SD of the rate of change.

Supplemental Table S3. Characteristics of patients included in clustering by glucotype during the first year of type 1 diabetes (n= 65, 168 visits)

	Group 1	*Group 2*	*Group 3*	*Group 4*	*p-value**
Characteristics	*(N=39)*	*(N=37)*	*(N=66)*	*(N=27)*	
Distribution					
Age — years	12.8 ± 2.9	10 ± 6.5	9.8 ± 4.6	8.9 ± 2.5	0.0001†
Sex — Male no. (%)	23 (59)	17 (45.9)	38 (57.6)	8 (29.6)	0.053‡
Pubertal — no. (%)	33 (84.6)	23 (62.2)	26 (39.4)	6 (22.2)	1.8e-07‡
BMI (Z-score)	0.3 ± 0.7	0.1 ± 1.1	0.1 ± 1	0.7 ± 1.1	0.054†
Glycemic control§					
HbA_{1C} — % [mmol/mol]	5.6[38] ± 0.4[4]	6.4[46] ± 0.5[6]	6.5[48] ± 0.6[6]	8.1[65] ± 0.8[9]	4.1e-35†
Insulin doses (IU/kg/day)	0.5 ± 0.2	0.5 ± 0.2	0.7 ± 0.3	0.7 ± 0.2	1.4e-11‖
$IDAA_{1C}$	7.5 ± 0.8	8.2 ± 0.9	9.5 ± 1.5	11 ± 1	1.5e-25†
Fasting and stimulated C-peptide¶					
$CPEP_{BASAL}$ (pmol/mL) — n=81	0.3 ± 0.2	0.4 ± 0.2	0.1 ± 0.2	0.1 ± 0.1	1.4e-05‖
$CPEP_{EST}$# (pmol/mL) — n=81	0.6 ± 0.2	0.7 ± 0.3	0.4 ± 0.2	0.3 ± 0.1	2.9e-08‖
Global control§					
Mean glucose (mg/dL)	104 ± 4	138 ± 17	135 ± 18	198 ± 25	6.4e-49†
Hypoglycemia§					
Below 70 mg/dL — %	14 ± 7	5 ± 2	14 ± 7	8 ± 6	2.5e-14‖
Below 54 mg/dL — %	4 ± 2	2 ± 0	6 ± 5	4 ± 3	1.1e-13‖
LBGI	3.9 ± 1.1	1.9 ± 0.5	3.7 ± 2	2.2 ± 1.2	4.9e-15‖
Time in range§					
In range 63-140 mg/dL — %	83 ± 6	60 ± 14	55 ± 11	27 ± 10	1.3e-45†
In range 70-180 mg/dL — %	86 ± 7	80 ± 8	67 ± 9	42 ± 9	2e-49†
Hyperglycemia§					
Above 180 mg/dL — %	5 ± 2	20 ± 9	23 ± 10	55 ± 13	6.8e-27‖
Above 250 mg/dL — %	2 ± 0	5 ± 2	8 ± 4	28 ± 9	8.8e-28‖
HBGI	1.8 ± 0.4	4.8 ± 1.9	5.7 ± 2.2	15.6 ± 4	1.6e-26‖
Total variability§					
Coefficient of variation — %	32 ± 5	32 ± 5	46 ± 7	47 ± 9	7.9e-22‖
ADRR	22 ± 5	30 ± 5	41 ± 8	58 ± 8	8e-51†

Within-day variability[§]					
SD_{ROC} (mg/dL)	1.9 ± 0.2	2.3 ± 0.2	2.6 ± 0.4	3.3 ± 0.5	3.1e-23[‖]
CONGA	36 ± 7	56 ± 10	73 ± 14	106 ± 18	4e-51[†]
Between-day variability[§]					
Interquartile range (mg/mL)	4 ± 0	4 ± 0	4 ± 0	5 ± 0	5.4e-49[†]
MODD	27 ± 6	43 ± 8	56 ± 1	83 ± 15	6.4e-51[†]
Combination score[§]					
COGI	66 ± 16	79 ± 6	52 ± 11	47 ± 10	9.1e-27[†]

Legend: Plus-minus values are means ± SD. Percentages may not total 100 because of rounding. * p-value calculated between all clustering groups, results were considered as significant when under 0.05. [†] Linear regression; [‡] Chi-square; [§] parameters evaluated at +3, +6, +9, +12 months after diagnosis; [‖] Kruskal-Wallis; [¶] parameters evaluated at +3 and +12 months after diagnosis; [#] calculated as described in Wentworth *et al*. Abbreviations: ADRR, average daily risk range; COGI, composite continuous glucose monitoring index; CONGA, continuous overall net glycemic action; HBGI, high blood glucose index; HbA_{1C}, Hemoglobin A1C; LBGI, low blood glucose index; MDI, Multiple Daily Injection; MODD, mean of the daily differences; SD_{ROC}, SD of the rate of change.

Supplemental Figure S1

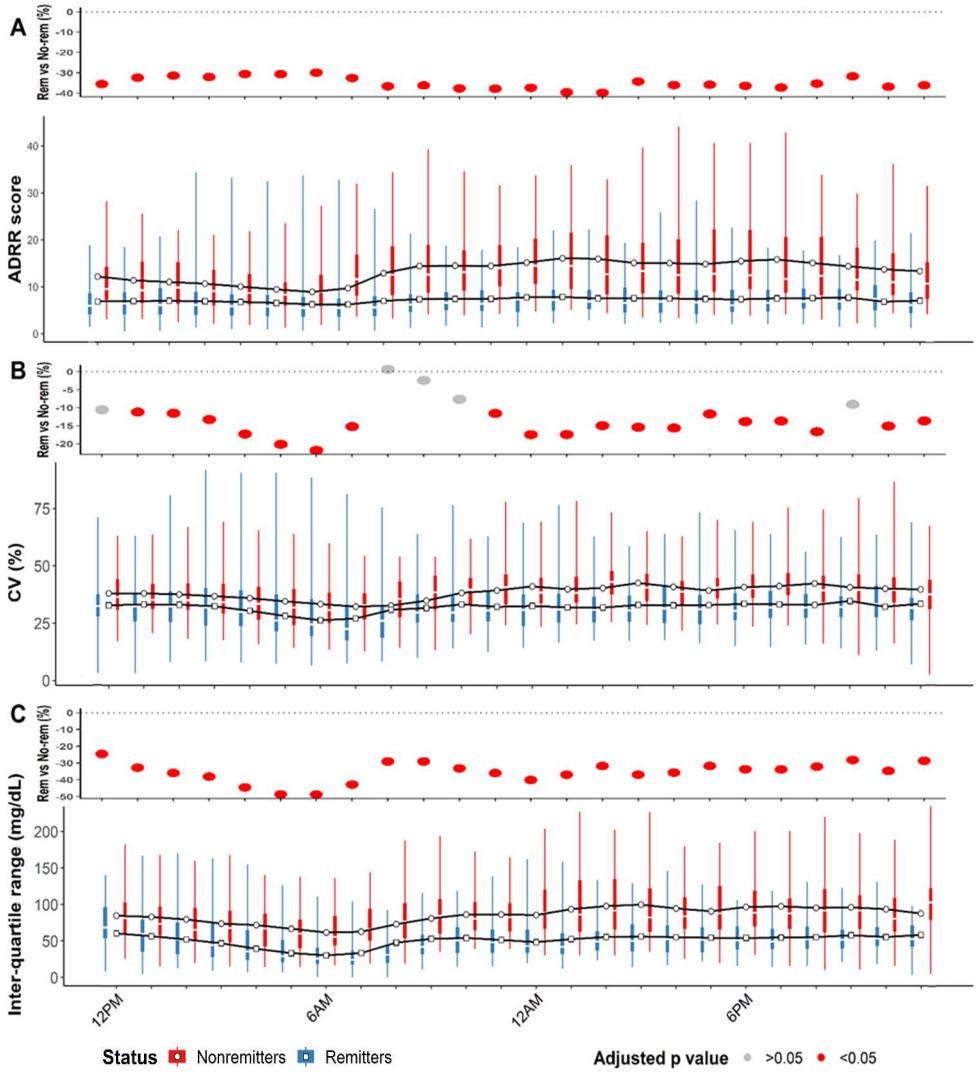
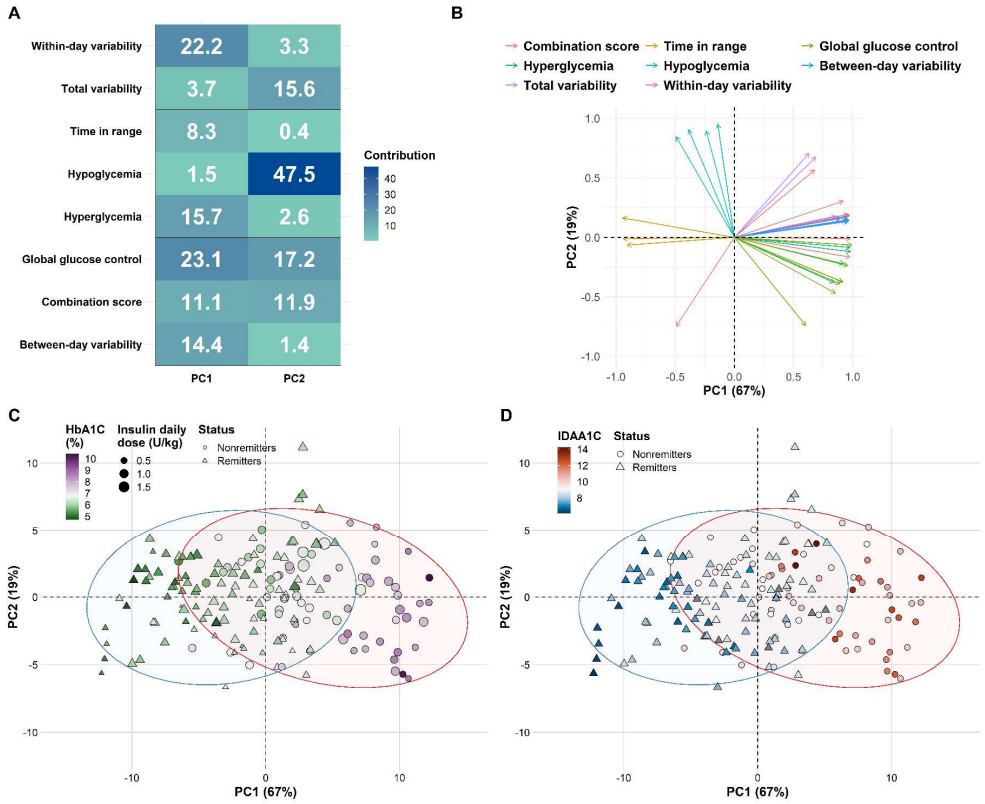


Figure S1: The daily patterns of hypo- and hyperglycemia risk score **(A)**, global **(B)** and between-day **(C)** glycemic variability regarding to the remission status evaluated during the first year of type 1 diabetes. Routine clinical parameters and CGM metrics were obtained at +3, +6, +9, +12 months after diagnosis. **(A)** The circadian evolution of the Average Daily Risk Range (ADRR) **(B)** The circadian evolution of the coefficient of variation (CV). **(C)** The circadian evolution of inter-quartile range. Box plots display the median, 25th and 75th percentiles, and ranges in remitters *(blue*) and nonremitters *(red).* Black lines represent the mean value of

the parameter in remitters (*white squares*) and nonremitters (*white dots*). The inset panels above each glycemic metric figure represent the daily variation in the amplitude of differences based on a linear mixed model (i.e. percentage above 0% [*dashed black line*] defining higher values in remitters, percentage below 0% defining lower values in remitters). The significance level of the differences is represented by the color of the points as follows: *red dots* correspond to p value <0.05 and *gray dots* represent p value >0.5.

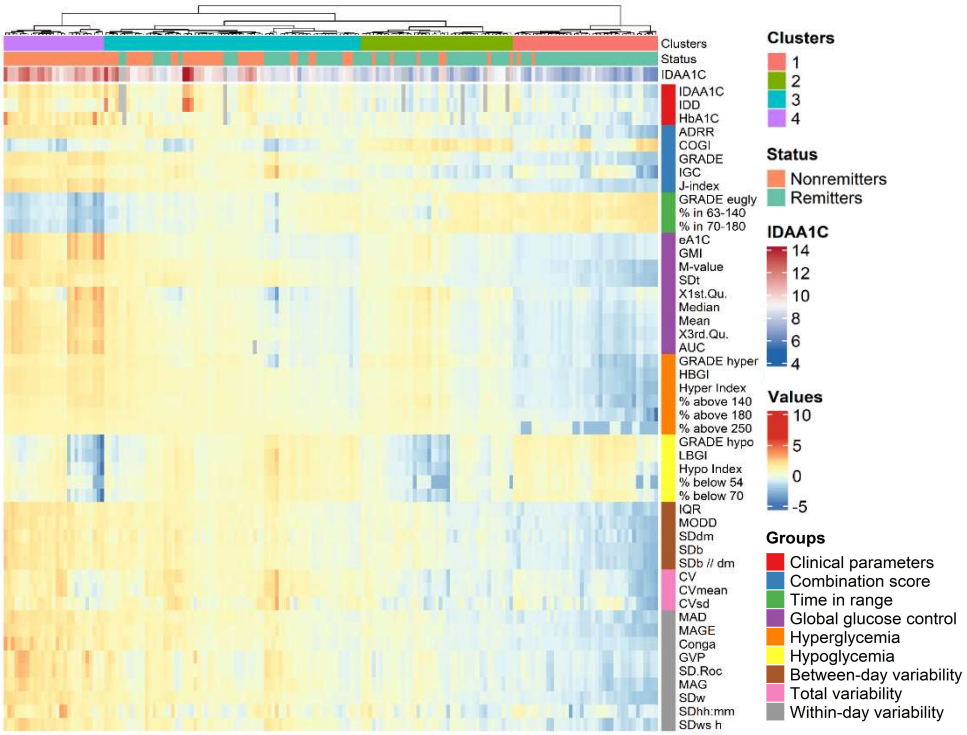
Supplemental Figure S2



Supplemental figure S2: Representation of new-onset type 1 diabetes patients during the first year of diabetes based on CGM metrics and according to their remission status. The PCA plots **(C-D)** show the patients at a defined time point as points, in two combined measure dimensions (*PC1* and *PC2*) that capture the contribution of most CGM metrics variation across the population and illustrated regarding their parameters group **(A-B)**. **(A)** Correlation plot showing the sum of

glycemic metrics contribution to PCA, according to their CGM parameter groups. **(B)** Graphical visualization of the contribution of the most influential CGM metrics (*i.e.* square cosine >0.75) to PC1 according to their group of CGM parameters (*arrow color*). The *length* and *direction* of each arrow correspond to the respective contributions of the CGM metrics to PC1 (*horizontal direction*) and PC2 (*vertical direction*). **(C)** The determinants of $IDAA_{1C}$ score were decomposed into HbA_{1C} levels (%), using continuous colored gradient (*purple-green*) with HbA_{1C} = 6.5% in *white*, and daily doses of insulin (U/kg), represented by the size of dots. Ellipses define the regions that contain 95% of all patients according to their remission status (remitters = *blue circle*, nonremitters = *red circle*) **(D)** Color scale represents $IDAA_{1C}$ score where remitters (i.e. $IDAA_{1C}$<9) are represented in *blue*, nonremitters in *red* ($IDAA_{1C}$>9) and $IDAA_{1C}$=9 in *white*. Ellipses define the regions that contain 95% of all patients according to their remission status (remitters = *blue circle*, nonremitters = *red circle*). Abbreviations: PCA = principal component analysis; CV = coefficient of variation; CGM = continuous glucose monitoring; $IDAA_{1C}$ = insulin dose-adjusted A_{1C}.

Supplemental Figure S3



Supplemental Figure S3: Hierachical clustering heatmap based on forty-six CGM metrics and clinical parameters (*rows*) evaluated during the first year of type 1 diabetes using Euclidian distances. Scaled and centred *values* are categorized as high *(red*), medium *(yellow*) and low *(blue*). The bars above the panel represent the characteristics of the patient including his *cluster* (group 1 = *green*, group 2 = *red*, group 3 = *light blue*, group 4 = *purple*), *remission status* (nonremitters = *orange*, remitters = *blue-green*), *and* $IDAA_{1C}$ *score* (>9 = *red,* 9 = *white*, <9 = *blue*). CGM metrics were subclassified in different *groups*. Abbreviations: CGM = Continuous Glucose Monitoring; $IDAA_{1C}$ = insulin dose-adjusted A_{1C}.

3.5 Paper II: Post-Hypoglycemic Hyperglycemia Are Highly Relevant Markers For Stratification Of Glycemic Variability and Remission Status Of Pediatric Patients With New-Onset Type 1 Diabetes.

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Key words: Biomarkers, Children, Clustering, Continuous glucose monitoring, Glycemic variability, Hypoglycemia, Insulin dose-adjusted A1C, Partial Remission, Type 1 Diabetes, Therapeutic management.

3.5.1 Abstract

Aims: To evaluate whether Post-Hypoglycemic Hyperglycemia (PHH) parameters correlated with glycemic homeostasis during the first year after type 1 diabetes onset and helped to distinguish pediatric patients undergoing partial remission or not.

Methods: In the GLUREDIA study, longitudinal values of clinical parameters, continuous glucose monitoring metrics and residual beta-cell secretion from children with new-onset type 1 diabetes were analyzed on a one-year basis. PHH parameters were calculated using an in-house algorithm. Cross-sectional correlations between PHH parameters and glycemic homeostasis markers were performed using adjusted mixed-effects models.

Results: PHH parameters were strong markers to differentiate remitters from non-remitters with PHH/Hyperglycemia duration ratio being the most sensitive. Interestingly, PHH_{AUC} moderately correlated with clinical parameters) and continuous glucose monitoring metrics while showing weaker inverse correlations with residual beta-cell secretion. Furthermore, combination of PHH parameters identified groups of patients that might benefit from distinct therapeutic management. Finally, patient classification into four glucotypes, as previously described, independently validated PHH parameters as reliable markers of glycemic homeostasis and improved the segregation of patients with intermediate values of $IDAA_{1C}$ and $CPEP_{EST}$.

Conclusion: PHH parameters are new minimal-invasive markers of partial remission and glycemic homeostasis during the first year of type 1 diabetes that allow patient-specific therapeutic management.

3.5.2 Introduction

Type 1 diabetes mellitus is characterized by a progressive decline in beta-cell mass, resulting in a clinical state of insulinopenia when beta-cell function drops below twenty percent (1), which defines the clinical disease onset. From that moment, patients with type 1 diabetes rely on a combination of exogenous insulin administration, healthy diet and regular physical activity to achieve optimal glycemic control (2–4). Immediately after disease onset and insulin therapy initiation, the majority of patients (occurrence rate: [40–75%]) experience a period of partial remission defined by the coexistence of low levels of glycemic variability and reduced exogenous insulin requirements (4). However, after partial remission, a progressively growing dependence on exogenous insulin induces an increased glycemic variability and the difficulty for patients to avoid hypoglycemia. Indeed, despite major improvements in diabetes management (5), nearly half of type 1 diabetes patients do not reach recommended therapeutic targets (6). Also, under classical intensive insulin therapy, an incompressible hypoglycemia frequency (estimated at 5-15% of total glucose values (7)) is unavoidable to maintain mean glycemia within targets.

Hypoglycemia is the most common complication in type 1 diabetes patients (7) and corresponds to an inadequacy between insulin substitution, insulin needs and carbohydrate intake with a consecutive drop of glycemia (i.e., below 60 mg/dL). Hypoglycemic events are either asymptomatic or associated with mild-to-severe clinical manifestations, such as convulsions and/or loss of consciousness (8,9). Though rarely life-threatening, children experience on average three symptomatic hypoglycemic events per week

that commonly require external intervention (i.e., providing oral carbohydrates or glucagon analogs) (7). Additionally, as hypoglycemia is an acute, stressful and unpleasant event for both the child and the parents, these events may lead to excessive carbohydrate intake followed by acute hyperglycemia and, more globally, increased glycemic variability (10). This highlights hypoglycemia as a potential trigger of hyperglycemic excursions and the need for therapeutic education focusing on individual profiles of glycemic variation.

In patients with type 1 diabetes, hypoglycemic events were associated with increased oxidative stress (11–13) that participates in the development of microvascular complications (e.g., diabetic retinopathy and nephropathy) (14,15). This was clinically demonstrated by Ceriello et al. who observed increased expression of oxidative stress markers in patients experiencing hypoglycemia followed by hyperglycemia, while the same markers remained unchanged when a normoglycemic state was maintained (16). These results suggest a link between inadequate carbohydrate intake during hypoglycemia and the development of diabetes complications.

Based on these observations, our team recently revised the concept of post-hypoglycemic hyperglycemia (PHH) and investigated their influence on diabetes control (EPHICA study) (17). PHH was defined as a hypoglycemia (i.e., glycemia <60 mg/dL) followed, within 2 hours, by hyperglycemia (i.e., glycemia >160 mg/dL) **(Fig. 1).** In pediatric patients with longstanding type 1 diabetes (i.e., clinical onset >1 year), these glycemic patterns represented more than a third of time spent in hyperglycemia for nearly 15% of patients and displayed strong correlations with markers of glycemic variability (17).

While current beta-cell function markers lack reliability in reflecting the extents of glycemic homeostasis in patients with type 1 diabetes, our recent results support PHH as reliable and minimal-invasive markers of glycemic homeostasis that may help individualizing therapeutic interventions (18).

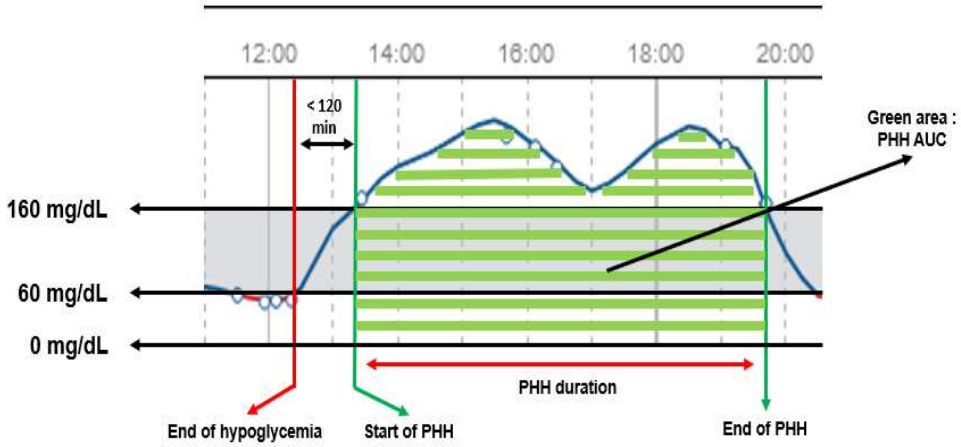

Figure 1: Graphical representation of a continuous analysis of subcutaneous glucose (vertical axis) over a period of 9 hours (horizontal axis). The normoglycemia range is 60-160 mg/dL. In this example: end of hypoglycemia at 00:20 p.m., onset of PHH at 01:20 p.m., end of PHH at 07:45 p.m. Hyperglycemia occurred less than 2 hours after the end of hypoglycemia; PHH lasted for 6 hours and 25 minutes. The green area is the PHH_{AUC}.

The objectives of our GLUREDIA study were to characterize PHH events in a cohort of pediatric patients with new-onset type 1 diabetes and investigate whether PHH parameters correlated with glycemic homeostasis including clinical parameters (e.g., HbA_{1C}, total daily dose of insulin, Insulin-Dose Adjusted A_{1C}), continuous glucose monitoring metrics or residual beta-cell secretion (i.e., $CPEP_{EST}$). Also, we evaluated whether these PHH parameters supported the clinical heterogeneity suggested by the newly identified glucotypes during the first year after T1D onset.

3.5.3 Material and methods

Study design & participants

GLUREDIA is a subsidiary study of the multicentric DIATAG study that was previously described (19). Briefly, DIATAG included new-onset pediatric patients with type 1 diabetes aged between 6 months and 17 years old. type 1 diabetes was diagnosed using International Society for Pediatric and Adolescent Diabetes (ISPAD) criteria (1) and patients were positive for at least one anti-islet autoantibody (i.e., anti-insulin, anti-protein tyrosine phosphatase, anti-glutamic acid decarboxylase, anti-Zinc transporter 8). All participants and their parents gave their written consent prior to enrolment in the study. The protocol was approved by the seven participating ethical committees (Comité d'Ethique Hospitalo-Facultaire of CUSL (2018/04DEC/462) and is registered in www.clinicaltrial.gov (NCT04007809). Exclusion criteria are described elsewhere (19). All participants were without significant comorbidities at inclusion (i.e., high blood pressure (>P95), proteinuria (>0.15 g/L) or body mass index [BMI] Z-score >+3 DS according to Cole et *al*. (20)).

A data array was collected at diagnosis and included demographics of the patient (i.e., age at diagnosis, sex, pubertal status, weight, height and BMI) and diabetes characteristics (i.e., presence of ketoacidosis at diagnosis, anti-islet antibodies, insulin regimen). From diagnosis, clinical parameters (i.e., Insulin-Dose Adjusted. A1C [$IDAA_{1C}$], HbA_{1C}, total insulin daily dose [TDD]) and raw continuous glucose monitoring metrics were collected at each outpatient clinical visit (i.e., every 3 months) for 1 year. All data were

gathered inside the Research Electronic Data Capture (REDCap) system provided by Vanderbilt University (Nashville, USA) and hosted at Cliniques universitaires Saint-Luc.

Continuous glucose monitoring metrics analysis and PHH detection

Raw continuous glucose monitoring metrics from a 90-day interval were extracted at each outpatient clinical visit from various continuous glucose monitoring devices (i.e., Freestyle Libre®, Abbott; Dexcom®, Dexcom; EnliteTM, Medtronic MiniMed). Raw glycemic data were pre-processed using the R (R Core Team (2021)) statistical package cgmanalysis. Data quality check and calculation of a panel of forty-six continuous glucose monitoring metrics were performed using the Iglu statistical package.

For PHH detection, raw continuous glucose monitoring metrics were aggregated on T-SQL™ (Azure SQL Instance) and, quality-checked and optimized using in-house PHH detection algorithm implemented on C# 7.3 using .NET framework. continuous glucose monitoring metrics exhibiting a time lapse above 45 minutes between consecutive glycemic values were excluded from the analysis. Glycemic values below 20 mg/dL or displaying a glycemic change above 100 mg/dL in less than 5 minutes were considered as artifacts and further excluded from the analysis. Manual inspection of PHH patterns for algorithm accuracy-check was performed using PowerBI (Microsoft™). PHH event was defined as a hypoglycemic episode (i.e., interstitial glucose <60 mg/dL) followed, within two hours, by a hyperglycemic episode (i.e., interstitial glucose >160 mg/dL). The PHH event starts when a value above 160 mg/dL is detected within two hours after hypoglycemia. If a glycemic value below 160 mg/dL (e.g., 155 mg/dL) is

identified during the PHH event and followed, within a maximum of 15 minutes, by a glycemic value above 160 mg/dL; the PHH event continues. The ends when two consecutive glycemic values below 160 mg/dL (i.e., >15 minutes) are detected. The PHH duration corresponds to the difference in minutes between the start and the end of PHH. The area under the curve (AUC) of PHH was calculated using the irregular polygon rule (21) where PHH starts and ends are defined as y=0. The algorithm will be added to GitHub repository.

Partial remission

Partial remission was defined by $IDAA_{1C} = HbA_{1C} + (4 \times \text{insulin doses/kg/d})$ (22), where a score below 9 defines remitters and a score above 9 defines non-remitters.

Residual C-peptide secretion

Residual C-peptide secretion ($CPEP_{EST}$) was evaluated at +3 and +12 months after diagnosis. Stimulated C-peptide values were estimated using a mathematical formula described by Wentworth *et al* ($Log_e [CPEP_{EST} + 1] = 0.317 + 0.00956 \times BMI - 0.000159 \times duration + 0.710 \times FCP - 0.0117 \times FPG - 0.0186 \times HbA_{1C} - 0.0665 \times insulin$, where BMI is in kg/m^2, duration is in days, Fasting C-Peptide [FCP] is in nmol/l, Fasting plasma glucose [FPG] is in mmol/L, HbA_{1C} is in % and insulin is in IU/kg) (23). Fasten C-peptide and plasmatic glucose values were determined at the central laboratory of CUSL for all samples. C-peptide was measured using a two-site chemiluminescence immunoassay (LiaisonXL®, Diasorin, France).

Glucotypes

Patient glucotypes were recently described by our clinical research center using hierarchical clustering based on continuous glucose monitoring metrics and clinical parameters (19). Briefly, these glucotypes exhibited specific nychthemeral profiles of continuous glucose monitoring metrics and refined the current dichotomic definition of partial remission.

Statistical analyses

Most statistical analyses were performed R (R Core Team [2021]. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL [https://www.R-project.org/]). In the end, Roc analyses were done using SAS v.9.4 ([https://www.sas.com/en_us/home.html]). The statistical significance level used for all analyses was 0.05. PHH parameters were transformed using Box-Cox transformation when needed. Demographic and clinical data are reported as mean ± SD for continuous variables and as numbers and proportions for categorical variables. Comparisons between groups were performed using Student's t test, chi-square test and linear regression or their nonparametric equivalent (Mann–Whitney U test and Kruskal–Wallis test, respectively) as appropriate. P-values were adjusted for multiple testing with the Bonferroni method (24). Marginal R^2 (coefficient of determination) between PHH parameters and secretion, continuous glucose monitoring metrics and clinical parameters were calculated using generalized linear mixed models with R packages lme4 (25) and lmerTest

(26) to take into account multiple measurements from the same patient. Models included the methods as fixed effects and patient identity as a random intercept. Residuals were inspected for normality on Q-Q plots.

3.5.4 Results

Clinical and anthropometric characteristics of the GLUREDIA cohort

Seventy-one pediatric patients with new-onset type 1 diabetes were quarterly followed during the first year after diagnosis, corresponding to a total of 241 outpatient visits. Of these visits, 48 were excluded as they did not fulfill our pre-established quality criteria or had missing associated clinical data. Final analysis was performed on 193 clinical visits from 66 patients (i.e., 59 patients at +3, 48 patients at +6, 45 patients at +9 and 41 patients at 12 months after the diagnosis) representing a total of 1900000 interstitial glucose values. The baseline characteristics (i.e., at diagnosis) of the cohort are described in **Table 1**.

PHH are frequent during the first year after type 1 diabetes onset

PHH parameters were calculated at each time point from a 3-month continuous glucose monitoring metrics period and included the proportion of total duration of all PHH according to total time spent in hyperglycemia (PHH/Hyperglycemia duration ratio) and the hypoglycemia proportion followed by a PHH (PHH/Hypoglycemia frequency ratio). Of the 193 analyzed continuous glucose monitoring metrics, 174 (90%) exhibited at least one PHH event in the 3-month period. Globally, participants presented 0.19 (±0.20) PHH events/day that lasted on average 155 (±117) minutes corresponding to a mean PHH_{AUC} of 32023 (±30037). The PHH/Hyperglycemia duration ratio was 0.04 (±0.05) and the PHH/Hyperglycemia frequency ratio was 0.26 (±0.18), whereas the PHH/Hypoglycemia frequency ratio was 0.35 (±1.06) meaning that, in

average, about a third of hypoglycemia were followed by hyperglycemia in our patient cohort **(Table 1).**

Next, we investigated the circadian rhythm of the PHH, as physiological phenomena or behavioral aspects might influence the occurrence of these events (e.g., Somogyi effect (27), exogenous carbohydrate intake). We subdivided the whole day into four distinct periods: morning (5 a.m.; 10 a.m.; $morning_{5\text{-}10}$), day (10 a.m.; 4 p.m.; $day_{10\text{-}4}$), evening (4 p.m.; 10 p.m.; $evening_{4\text{-}10}$) and night (10 p.m.; 5 a.m.; $night_{10\text{-}5}$). $Morning_{5\text{-}10}$ accounted for the occurrence of the Somogyi effect, $day_{10\text{-}4}$ corresponded to the school period, $evening_{4\text{-}10}$ to the homestay period with parental control and $night_{10\text{-}5}$ to the period with lowest influence of exogenous insulin or carbohydrate intake. We observed a higher PHH frequency during the $day_{10\text{-}4}$ (0.14 PHH/$day_{10\text{-}4}$) and $evening_{4\text{-}10}$ (0.09 PHH/$evening_{4\text{-}10}$) than in the $morning_{5\text{-}10}$ (0.06 PHH/$morning_{5\text{-}10}$) and $night_{10\text{-}5}$ (0.04 PHH/$night_{10\text{-}5}$) ($p=2.2e^{-16}$). On the other hand, the PHH_{AUC} increased progressively according to the different daytime periods: it was the lowest in the $morning_{5\text{-}10}$ (28000 ± 67000), intermediate during the $day_{10\text{-}4}$ (35000 ± 49000) and the $evening_{4\text{-}10}$ (46000 ± 72000), and the highest at $night_{10\text{-}5}$ (60000 ± 59000) ($p<0.05$).

PHH are thus a common phenomenon in patients with *de novo* type 1 diabetes and demonstrate high inter-patient variability (i.e., high SD) for each global parameter (i.e., PHH frequency, PHH duration mean, PHH_{AUC}, PHH/Hyperglycemia duration ratio and PHH/Hypoglycemia frequency ratio). Evaluation of PHH across the day revealed that these occurred more

frequently during the day[10-4] while being of longer duration during the night[4-10].

Table 1: Cohort description and distribution of PHH parameters.

	Global	Remitters	Nonremitters	p-values
	(*n*= 193)	(*n*= 108)	(*n*= 85)	
Phenotypic characteristics				
Age – years (at diagnosis)	10.7 ± 3.4	11.3 ± 3.3	9.8 ± 3.3	0.05 †
Sex – Male no (%)	95 (50)	71 (66)	24 (30)	<0.001 ‡
Pubertal – no (%)	98 (52)	64 (59)	34 (42)	0.02 ‡
Parameters of glycemic homeostasis				
Clinical parameters				
Hb_{A1C} – %	6.6 ± 1	6 ± 0.5	7.3 ± 0.9	<0.001 †
$IDAA_{1C}$	9 ± 1.6	7.9 ± 0.70	10.4 ± 1.2	<0.001 †
Continuous glucose monitoring metrics				
CV – %	38.9 ± 8.6	36.1 ± 8.8	42.5 ± 6.5	<0.001 †
MODD – %	47.9 ± 18.6	38.2 ± 13.5	60.6 ± 16.5	<0.001 †
Meanglycemia – mg/dL	134.5 ± 30	118.5 ± 17.2	155.7 ± 30.3	<0.001 †
TIR_{70-180} – %	71.7 ± 15	79.4 ± 11.1	61.5 ± 13.4	<0.001 †
$TBR_{<70}$ – %	9.5 ± 7.5	10.8 ± 8.4	7.6 ± 5.8	0.005†
$TAR_{>180}$ – %	19.7 ± 16.2	10.8 ± 9	31.5 ± 16	<0.001 †

PHH parameters				
Frequency – no/day	0.19 ± 0.20	0.15 ± 0.19	0.25 ± 0.2	<0.001 ‖
PHH_{AUC}	32023 ± 30037	18895 ± 17387	48220 ± 34278	<0.001 ‖
PHH duration mean (minutes)	155 ± 117	105 ± 75	217 ± 130	<0.001 ‖
PHH/hyperglycemia duration ratio – no	0.04 ± 0.05	0.02 ± 0.02	0.07 ± 0.06	<0.001 ‖
PHH/hypoglycemia frequency ratio – no	0.35 ± 1.06	0.28 ± 1.02	0.44 ± 1.12	<0.001 ‖
Circadian rhythm of PHH frequency – no				
$Morning_{5-10}$	0.04 ± 0.07	0.04 ± 0.09	0.04 ± 0.06	>0.99 ‖
Day_{10-4}	0.18 ± 0.27	0.10 ± 0.26	0.26 ± 0.32	<0.001 ‖
$Evening_{4-10}$	0.11 ± 0.17	0.06 ± 0.10	0.17 ± 0.21	<0.001 ‖
$Night_{10-5}$	0.03 ± 0.05	0.01 ± 0.02	0.04 ± 0.06	0.07 ‖

Legend: Plus-minus values are means ± SD. Percentages may not total 100 due to rounding. Glycemic homeostasis markers and PHH parameters were evaluated at +3, +6, +9, +12 months after diagnosis. Differences between remitters and nonremitters were considered as significant when p-value was under 0.05. † student t-test; ‡ Chi-square; ‖ Linear mixed models using Satterthwaite t-test. PHH, post-hypoglycemia hyperglycemia; HbA_{1C}, Glycated hemoglobin; $IDAA_{1C}$, Insulin Dose-Adjusted A1C = HbA_{1C} +4x insulin doses/day/kg; CV, coefficient of variation for glucose; MODD, mean of daily differences; TIR_{70-180}, Time in range (70-180 mg/dL); $TBR_{<70}$, Time below the range (<70 mg/dL); $GIIH_{<54}$, Grade 2 hypoglycemia (<54 mg/dL); $TAR_{>180}$, Time above the range (>180 mg/dL); $Hyperglycemia_{>250}$, Hyperglycemia above 250 mg/dL; PHH_{AUC}: area under the curve of PHH; PHH/Hyperglycemia duration ratio, ratio between the total duration of PHH and the total duration of hyperglycemia; PHH/Hypoglycemia frequency ratio, ratio between daily frequency of PHH and daily frequency of hypoglycemia; $Morning_{5-10,}$ morning time between 5 am and 10 am; $Day_{10-4,}$ daytime between 10 am and 4 pm, $Evening_{4-10,}$ evening time between 4 pm and 10 pm; $Night_{10-5,}$ nightime between 10 pm and 5 am.

Partial remission is associated with fewer and lower PHH

We next investigated whether PHH parameters reflected the occurrence and intensity of partial remission in patients with new-onset type 1 diabetes. Among the 193 glucose records studied, 108 (56%) belonged to patients undergoing partial remission.

Remitters presented fewer PHH events (0.15 ± 0.19 *vs* 0.25 ± 0.2 PHH/day; p<0.001) and these were of shorter duration (105 ± 75 vs 217 ± 130 minutes; p<0.001) and with smaller AUC (18895 ± 17387 vs 48220 ± 34277; p<0.001) than the one observed in non-remitters. Also, remitters exhibited fewer hypoglycemia followed by a PHH (PHH/Hypoglycemia frequency ratio; 0.28 ± 1.02 vs 0.44 ± 1.12; p<0.001) concurring to a four time decrease in the percentage of time spent in hyperglycemia due to a PHH (PHH/Hyperglycemia duration ratio; 0.02 ± 0.02 vs 0.07 ± 0.06; p<0.001), as compared to non-remitters **(Table 1)**. While the biggest PHH_{AUC} were observed during the $night_{10\text{-}5}$ for both groups (p<0.001); remitters exhibited smaller PHH_{AUC} compared to non-remitters, regardless of the daytime periods. Paradoxically, there was no significant difference in PHH frequency in the $morning_{5\text{-}10}$ and during the $night_{10\text{-}5}$ between remitters and non-remitters (p>0.05), contrasting with the two other daytime periods ($day_{10\text{-}4}$ and $evening_{4\text{-}10}$) where PHH frequency was higher for non-remitters than for remitters (p<0.05).

Supporting the clinical utility of these measures, we generated ROC curves and calculated the threshold for each parameter that distinguished remitters from non-remitters. All studied PHH parameters were able to

predict the remission status of a given patient (all p-values<0.05) with PHH/Hyperglycemia duration ratio being the most sensitive parameter (ratio<0.02; sensibility=86% and specificity=68%) and PHH duration mean the most specific parameter (duration mean <132 min; sensibility=76% and specificity=74%).

Finally, investigating the correlations between the partial remission intensity (i.e., $IDAA_{1C}$ score) and PHH parameters, we found moderate and strong correlations respectively between $IDAA_{1C}$ and PHH frequency (R^2=0.10; p<0.001) or PHH_{AUC} (R^2=0.32; p<0.001) **(Table 2).** Notably, large variability in the PHH frequency was observed for intermediate $IDAA_{1C}$ values (i.e., in 8-10 range)**.**

High residual C-peptide secretion is associated with a reduction of PHH parameters

As PHH parameters overlapped for intermediate $IDAA_{1C}$ scores, we evaluated whether replacing this score by a residual beta-cell secretion marker (i.e., $CPEP_{EST}$) might improve differences in PHH parameters among both remission groups. The patients were classified into four groups according to their residual estimated C-peptide secretion (28): high (c-peptide>0.4 pmol/mL), intermediate (0.2 pmol/mL<c-peptide⩽0.4 pmol/mL), low (0.17 pmol/mL<c-peptide⩽0.2 pmol/mL), and undetectable (c-peptide⩽0.17 pmol/mL).

$CPEP_{EST}$ moderately correlated with PHH frequency (R^2=0.08; p=0.006), PHH_{AUC} (R^2=0.17; p=<0.001) and strongly with PHH/Hyperglycemia duration ratio (R^2=-0.29; p<0.001). In addition, no correlation was found

between residual $CPEP_{EST}$ secretion and PHH/Hypoglycemia frequency ratio (R^2=0.03; p=0.13). Interestingly, patients with high residual secretion exhibited fewer and shorter PHH events, contrasting with patients with low or undetectable residual secretion (p<0.001) **(Table 2).** Finally, we observed that patients with intermediate secretion tended to have high intra-group variability in PHH frequency. Thus, when evaluating partial remission based on $CPEP_{EST}$ and $IDAA_{1C}$ score, we observed a non-discriminative zone where patients did not present an unequivocal remission or non-remission status, and where continuous glucose monitoring metrics, including PHH parameters, largely overlapped.

Table 2: Linear mixed-models determination coefficients (r^2) between parameters of PHH and glucose homeostasis.

	PHH frequency	PHH_{AUC}	PHH duration mean	PHH/Hyperglycemia duration ratio	PHH/Hypoglycemia frequency ratio
CV	0.24***	0.22***	0.20***	0.50***	0.001^{ns}
$IDAA_{1c}$	0.10***	0.32***	0.34***	0.30***	0.10***
MODD	0.05**	0.43***	0.40***	0.45***	0.17***
$Hypoglycemia_{<60}$ frequency	0.19***	0.02^{ns}	0.03*	0.07***	0.12***
TIT_{63-140}	0.04**	0.40***	0.38***	0.30***	0.27***
TIR_{70-180}	0.06***	0.35***	0.35***	0.40***	0.18***
$TAR_{>180}$	0.01*	0.42***	0.41***	0.28***	0.24***
$CPEP_{EST}^{\#}$	0.08**	0.17***	0.15***	0.29***	0.03^{ns}

Legend: Marginal R-squared (coefficient of determination) were calculated using generalized linear mixed models. Results were considered as significant when p-

value was under 0.05. Glycemic homeostasis markers and PHH parameters were evaluated at +3, +6, +9, +12 months after diagnosis. PHH, post-hypoglycemia hyperglycemia; Frequency, quantity of PHH per day; $PHH_{AUC,}$ area under the curve of PHH; PHH/Hyperglycemia duration ratio, ratio between the total duration of PHH and the total duration of hyperglycemia; PHH/Hypoglycemia frequency ratio, ratio between daily frequency of PHH and daily frequency of hypoglycemia; $IDAA_{1C,}$ Insulin Dose-Adjusted A1C = HbA1C + 4 x insulin doses/day/kg; CV, coefficient of variation for glucose; MODD, mean of daily differences; TIR_{70-180}, Time in range; TIT_{63-140}, Time in target; $TAR_{>180}$, Time above the range; $CPEP_{EST}$, estimation of c-peptide secretion calculated as described in Wentworth *et al* (23). The level of significance of the correlations is represented after the regression coefficient as follows: nonsignificant (ns), $p<0.05$ (*), $p<0.01$ (**), $p<0.001$ (***).

PHH parameters correlate with glycemic homeostasis markers

As correlations between PHH parameters and beta-cell residual secretion were moderate, we investigated whether PHH parameters would better correlate with continuous glucose monitoring metrics including glycemic variability (i.e., coefficient of variation [CV], mean of daily differences [MODD]) and time spent within different glycemic ranges (i.e., Time in range [TIR_{70-180}, 70-180mg/dL], Time above the range [$TAR_{>180}$, >180mg/dL], hypoglycemia frequency [<60mg/dL]) (**Table 2**)**.**

CV moderately correlated with nearly all PHH parameters ($p<0.05$) except for PHH/hypoglycemia frequency ratio ($p>0.05$) (**Table 2**). Interestingly, patients with CV values >36% exhibited three times more PHH events than patients with CV <36%. Also, MODD showed the strongest correlation among all glycemic homeostasis markers with all PHH parameters ($R^2>0.17$), except for PHH frequency. TIR_{70-180} inversely correlated with PHH parameters. Interesting, $TAR_{>180}$ weakly correlated with

PHH frequency. Also, the hypoglycemia frequency best correlated with PHH frequency (R^2=0.19; p<0.001) **(Figure 2).**

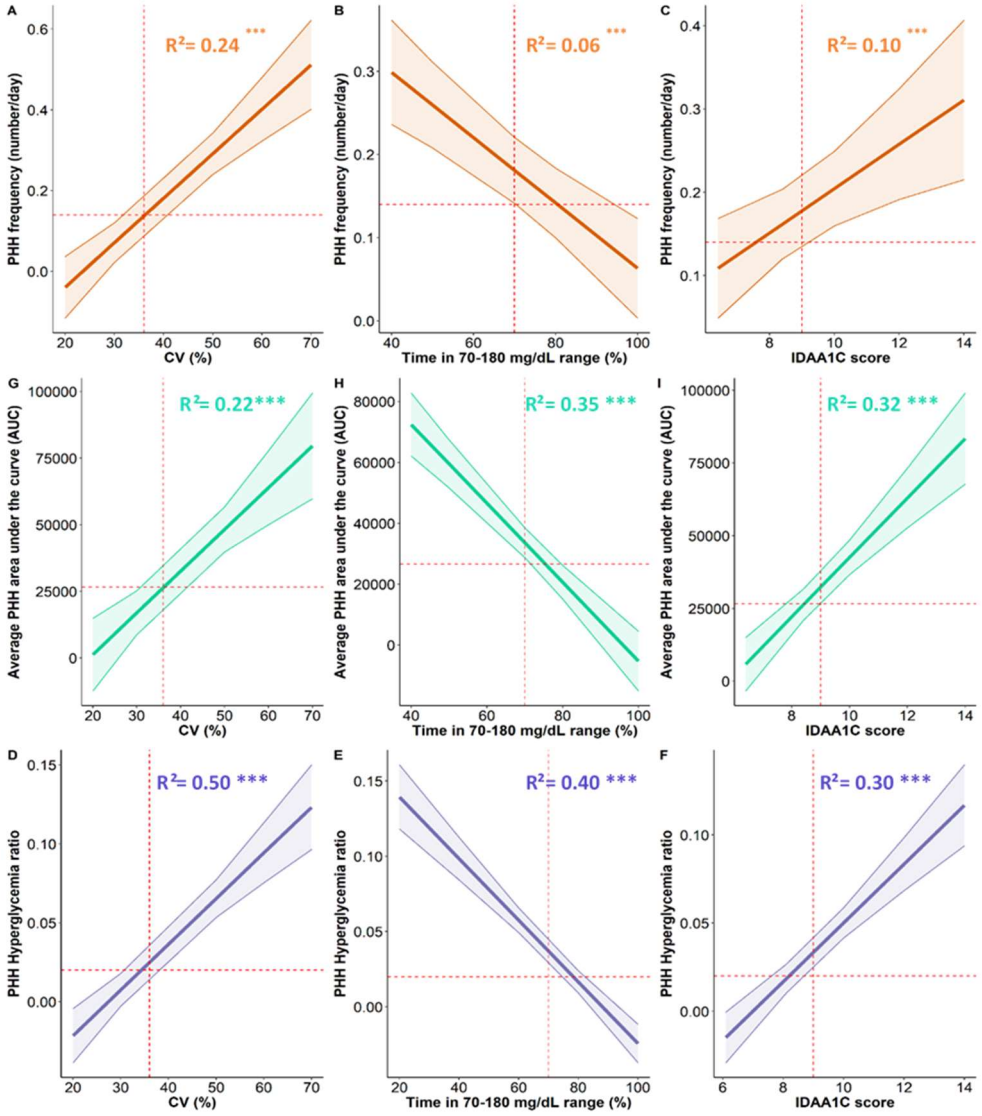

Figure 2: Determination coefficient of PHH frequency (A-C), PHH_{AUC} (D-F) and PHH/Hyperglycemia duration ratio (G-I) according to glycemic homeostasis markers (n=173). Parameters of PHH parameters and glycemic homeostasis markers were obtained at +3, +6, +9, and +12 months after the diagnosis. Marginal R-squared (coefficient of determination) were calculated using generalized linear

mixed models. The vertical red dashed lines represent specific thresholds of glucose homeostasis parameters (CV = 36%, time in 70-180 mg/dL range = 70% and $IDAA_{1C}$ score = 9). The horizontal red dashed lines represent specific status-related thresholds of PHH parameters (PHH frequency = 0.14, PHH_{AUC} = 26567 and PHH/Hyperglycemia duration ratio = 0.02). Panels **A-C** represent the regression results with 95% CI bands (shaded zone) between PHH frequency and CV (A), TIR (B) and $IDAA_{1C}$ (C). Panels **D-F** represent the regression results with 95% CI bands (shaded zone) between PHH_{AUC} and CV (D), time in 70-180 mg/dL range (E) and $IDAA_{1C}$ (F). Panels **G-I** represent the regression results with 95% CI bands (shaded zone) between PHH frequency and CV (G), time in 70-180 mg/dL range (H) and $IDAA_{1C}$ (I). Correlation coefficients (R) are shown according to the PHH frequency (**A-C**), PHHAUC (**D-F**) and PHH/Hyperglycemia duration ratio (**G-I**); Abbreviations: PHH frequency (number/day), PHH frequency; Average PHH area under the curve (AUC), PHH_{AUC}; PHH Hyperglycemia ratio, PHH/Hyperglycemia duration ratio; CV (%), coefficient of variation for glucose; Time in 70-180 mg/dL range, Time in range; IDAA1C score, Insulin Dose-Adjusted A1C = HbA_{1C} + 4 x insulin doses/day/kg. The level of significance of the correlations is represented after the regression coefficient as follows: nonsignificant (ns), $p<0.05$ (*), $p<0.01$ (**), $p<0.001$ (***).

Focusing on hypoglycemia and glycemic variability, patients were categorized into three different groups that demonstrated specific combinations of glycemic variability (i.e., CV) and PHH parameters (i.e., PHH_{AUC} and PHH/Hypoglycemia frequency ratio) (**Figure 3**). Group 1 corresponds to patients with stable type 1 diabetes (i.e., CV <36%) with small PHH/Hypoglycemia frequency ratio (<0.25) and very low PHH_{AUC} (<25000). Conversely, patients in Group 2 demonstrate highly variable PHH/Hypoglycemia frequency ratio (0.1-0.5) with low-to-intermediate PHH_{AUC} (<60000) and high glycemic variability (CV>36%). Finally, patients in Group 3 have very rare hypoglycemia that is usually followed by a PHH (PHH/Hypoglycemia frequency ratio [>0.4]) of high amplitude (PHH_{AUC} [>60000]). Interestingly, group 1 was mostly composed of remitters, and groups 2 and 3 from non-remitters. Finally, the temporal evolution of each

patient revealed that more than half of the patients stayed in the same group (53%) during the first year of type 1 diabetes while others switched from one group to another except from Groups 2 or 3 to Group 1.

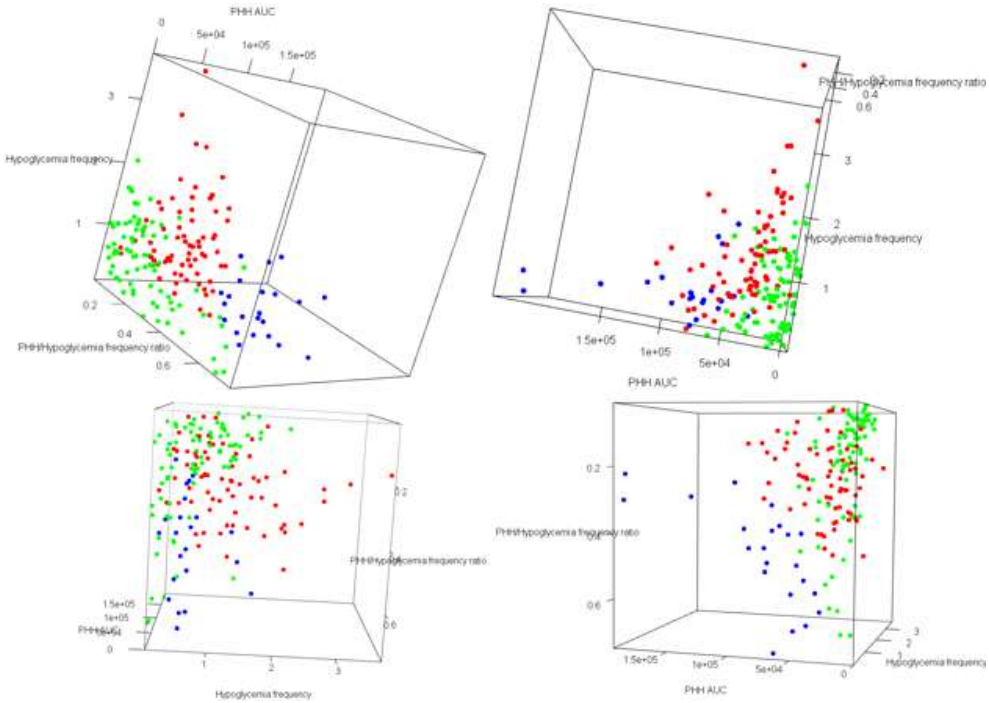

Figure 3: Hierarchical clustering in three target groups of patients according to PHH parameters. Multidimensional representation of PHH parameters according to the three target patient groups identified using hierarchical clustering. PHH parameters and glycemic homeostasis markers were obtained at +3, +6, +9 and +12 months after the diagnosis. Three-dimensional representation of PHH_{AUC}, frequency of hypoglycemia and PHH Hypo ratio. Each color of the dots is specific to a target group (green = Group 1, red = Group 2, blue = Group 3).

Patient glucotypes identify patients at risk for PHH.

To better characterize patients with non-discriminative indexes of glycemic homeostasis markers (i.e., intermediate $CPEP_{EST}$ secretions and $IDAA_{1C}$ scores), we studied PHH parameter distribution in the four distinct

glucotypes that were previously described by our team in patients during the first year after type 1 diabetes onset (19).

Glucotypes 1 and 2 demonstrated very low PHH duration mean representing a low PHH/Hyperglycemia duration ratio (< 0.01). While PHH_{AUC} of glucotype 3 remained similar to glucotype 2, we observed a major increase in PHH frequency and a concomitant rise in PHH/Hyperglycemia duration ratio in glucotype 3. Finally, contrasting with other glucotypes, glucotype 4 demonstrated the highest PHH/Hyperglycemia duration ratio (0.1 ± 0.08). Notably, glucotypes 2 and 4 were characterized by high PHH/Hypoglycemia frequency ratio though high variability could be observed between the patients (i.e., respectively 0.61 ± 0.23 and 0.77 ± 0.38) **(Figure 4)**.

Circadian PHH analysis showed an increase of PHH frequency in glucotypes 3 and 4 with highest differences being observed during the $day_{10\text{-}4}$ and $evening_{4\text{-}10}$ ($p<0.05$). Interestingly, PHH frequency in the $morning_{5\text{-}10}$ did not differ across glucotypes ($p>0.05$). Moreover, largest PHH_{AUC} were observed during the $night_{10\text{-}4}$ ($p<0.001$) regardless of glucotypes with patients in glucotype 1 experiencing the smallest PHH_{AUC}.

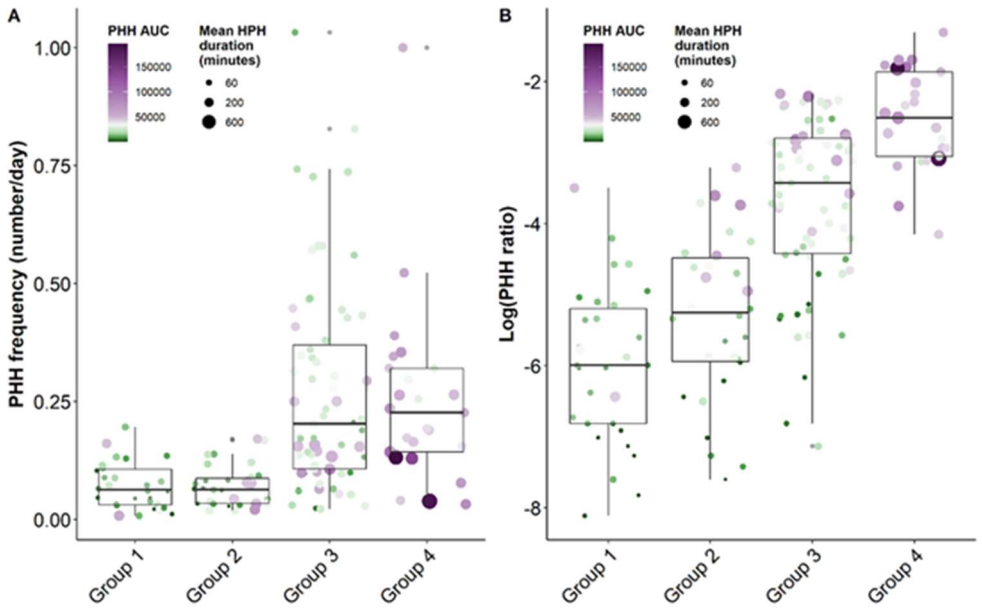

Figure 4: Distribution of PHH parameters according to the glucotypes (19)**.** PHH parameters and glycemic homeostasis markers were obtained at +3, +6, +9 and +12 months after the diagnosis. (A) Boxplots of PHH frequency according to glucotypes (group 1-4). (B) Boxplot of PHH/hyperglycemia duration ratio in log2 base according to glucotypes (group 1-4). Size of the dots corresponds to the PHH duration mean. Color of the dots represents levels of PHHAUC, using continuous colored gradient scale (green-purple) with PHHAUC = 26567 in white. Abbreviations: PHH frequency, quantity of PHH per day; PHH AUC, area under the curve of PHH; Mean HPH duration, average duration of a PHH; Log (PHH ratio), logarithm function of ratio between the total duration of PHH and the total duration of hyperglycemia.

3.5.5 Discussion

Partial remission reflects a transient recovery of beta-cell function with increased insulin secretion (29) and improved peripheral insulin sensitivity (30,31), leading to decreased dependence on exogenous insulin and optimal glycemic control (e.g., $TIR_{70\text{-}180}$, glycemia variability) (1). When beta-cell function further declines, insulin requirements and glycemic variability increase, corresponding to the end of partial remission and an increased hypoglycemic risk. Current definitions and biomarkers used to identify partial remission (i.e., residual secretion (28,30) or $IDAA_{1C}$ score (22)) either imply invasive blood sampling or present several limitations to describe the evolution of glycemic homeostasis (e.g., hypoglycemia, glycemic variability, insulin sensitivity) (18). In this context, there is a need for minimal-invasive reliable markers that allow the characterization and stratification of patients with type 1 diabetes based on their glycemic status.

In our GLUREDIA study, we extensively characterized PHH, a new marker of glycemic variability, in new-onset type 1 diabetes patients. Using 12-week continuous glucose monitoring metrics, we identified PHH parameters (e.g., PHH duration mean and PHH/hyperglycemia duration ratio) as both highly sensitive and specific markers to differentiate between patients undergoing remission or not. Furthermore, we established clinically-relevant segregation thresholds for each of them. Also, most PHH parameters demonstrated high variability across the day being the highest during the $day_{10\text{-}4}$ and $evening_{4\text{-}10}$. This suggested that reduced residual beta-cell function in combination with behavioral habits might be major triggers of PHH events. Finally, PHH parameters exhibited strong correlations with

continuous glucose monitoring metrics (i.e., $TIR_{70\text{-}180}$, $TAR_{>180}$, CV) while weakly-to-moderately correlating with residual beta-cell secretion.

Integrating previously described glucotypes (19), we showed that PHH parameters mirrored the progressive increase of glycemia variability across the various glucotypes (**Figure 4**). Interestingly, refining the ROC curves analysis, most false positive and negative patients had an $IDAA_{1C}$ score in the 8-10 range though distinctively distributing across glucotypes 2 and 3 that were previously described by our team (19). This observation independently validates our previous results, further confirms PHH parameters as reliable markers of glycemic homeostasis and supports recent evidence that partial remission should be considered as a continuum rather than a dichotomic phenomenon (19).

As previously mentioned, glycemic variability and hyperglycemia are both independent predictors of micro and macro-vascular complications through protein glycation (AGE) and oxidative stress (32,33). Paradoxically, continuous glucose monitoring metrics are not integrated into the main scores defining partial remission. In our study, most PHH parameters demonstrated to be strong markers of continuous glucose monitoring metrics (e.g., CV and MODD) (**Figure 2, Table 2**). Going further, our results support the clinical validity of the diabetes stability definition based on CV as a steeper increase of PHH parameters values was observed from the value of 36% (34). Moreover, Cerriello et al. showed that PHH occurrence was an independent risk factor of oxidative stress. Indeed, patients with type 1 diabetes presenting PHH displayed a pejoration of endothelial function, an

increase in inflammation and an increase of oxidative stress markers in an ischemic-reperfusion-like effect (16). These findings together reinforce PHH parameters as reliable and independent markers of glycemic homeostasis and a potential predictor of type 1 diabetes-related chronic complications.

Carbohydrate intake and management of hypoglycemia influence glycemic control in a patient-dependent way (35). Indeed, in our study, PHH principally occurred during the $day_{10\text{-}4}$ and $evening_{4\text{-}10}$ when the parents and/or the child are active and may strongly influence the glycemic control by some avoidable. Following this idea, the combination of PHH parameters (PHH_{AUC} and PHH/Hypoglycemia frequency ratio) and hypoglycemia frequency subdivided our type 1 diabetes patient cohort into three subgroups. Among these groups, the majority of patients in Group 2 frequently overtreated their hypoglycemia when trying to reach a normoglycemic state resulting in short and frequent PHH, and consequently high glycemic variability. Therefore, therapeutic education focusing on the management of hypoglycemia (7) and targeting a PHH/Hypoglycemia frequency ratio below 0.25 may considerably improve disease control in these patients, by preventing further PHH events with reduced glycemic variability.

3.5.6 Conclusion

Our study deepened the characterization of PHH events during the first year of type 1 diabetes, which revealed to be recurrent in our pediatric cohort, especially when diabetes was poorly controlled. PHH parameters (e.g., PHH_{AUC} and PHH/hyperglycemia duration ratio) allowed sensitive and specific distinction between remitters and non-remitters using a poorly invasive method. Also, PHH parameters independently supported partial remission as a continuum of dysglycemia, with a longitudinal increase along the four described glucotypes. Moreover, PHH_{AUC} and PHH/hyperglycemia duration ratio demonstrated to be reliable markers of glycemic homeostasis showing moderate-to-strong correlations with most glycemic control parameters (i.e., $TIR_{70\text{-}180}$, $TAR_{>180}$, CV) though only weak-to-moderate correlations were found with residual beta-cell secretion. Finally, the circadian analysis of PHH events highlighted the role of exogenous carbohydrate intakes in glycemic variability where the combination of PHH/hypoglycemia frequency ratio and PHH_{AUC} segregated patients into three groups that might foster patient-specific interventions. We believe that integrating PHH parameters in continuous glucose monitoring reports will raise awareness of the hypoglycemia overtreatment and provide an additional tool for physicians to improve the individualization of their educational interventions.

The principal strength of our multicentric and pediatric study relies on the cross-sectional data analysis that integrates complementary markers of glycemic homeostasis during the first year of type 1 diabetes. In addition, the establishment of PHH parameters thresholds allows an easy, reliable and

poorly invasive determination of the remission status and assessment diabetes control using freely-available algorithm.

Our study was also limited by the cross-sectional analysis of all three parameters (i.e., clinic, secretion and continuous glucose monitoring metrics) that were only available for a subset of patients (i.e., 70%). Also, the sensor manufacturer may influence the data though most of our dataset (i.e., >90%) obtained from Freestyle Libre®. Furthermore, the small numbers of patients using pump insulin delivery system did not allowed us to perform subanalysis according to the insulin regimen (i.e., multiple daily injections vs pump). Finally, data including the type of rapid insulin analog were not collected and thus could not be analyzed.

3.5.7 References

1. Couper JJ, Haller MJ, Greenbaum CJ, Ziegler AG, Wherrett DK, Knip M, et al. ISPAD Clinical Practice Consensus Guidelines 2018: Stages of type 1 diabetes in children and adolescents. Pediatr Diabetes. 2018;19:20–7.

2. Danne T, Phillip M, Buckingham BA, Jarosz-Chobot P, Saboo B, Urakami T, et al. ISPAD Clinical Practice Consensus Guidelines 2018: Insulin treatment in children and adolescents with diabetes. Pediatr Diabetes. 2018;19(July):115–35.

3. Smart CE, Annan F, Bruno LPC, Higgins LA, Acerini CL. Nutritional management in children and adolescents with diabetes. Pediatr Diabetes. 2014;15(SUPPL.20):135–53.

4. Zhong T, Tang R, Gong S, Li J, Li X, Zhou Z. The remission phase in type 1 diabetes: Changing epidemiology, definitions, and emerging immuno-metabolic mechanisms. Diabetes Metab Res Rev. 2020;36(2):0–2.

5. Prahalad P, Tanenbaum M, Hood K, Maahs DM. Diabetes technology: improving care, improving patient-reported outcomes and preventing complications in young people with Type 1 diabetes. Diabet Med. 2018;35(4):419–29.

6. Maiorino MI, Signoriello S, Maio A, Chiodini P, Bellastella G, Scappaticcio L, et al. Effects of continuous glucose monitoringonmetricsofglycemic control in diabetes: A Systematic Review with Meta-analysis of Randomized Controlled Trials. Diabetes Care. 2020;43(5):1146–56.

7. Abraham MB, Jones TW, Naranjo D, Karges B, Oduwole A, Tauschmann M, et al. ISPAD Clinical Practice Consensus Guidelines 2018: Assessment and management of hypoglycemia in children and adolescents with diabetes. Pediatr Diabetes. 2018 Oct 1;19:178–92.

8. Lavens A, Nobels F, De Block C, Oriot P, Verhaegen A, Chao S, et al. Effect of an Integrated, Multidisciplinary Nationwide Approach to

Type 1 Diabetes Care on Metabolic Outcomes: An Observational Real-World Study. Diabetes Technol Ther. 2021 Aug 1;23(8):565–76.

9. Maahs DM, Hermann JM, DuBose SN, Miller KM, Heidtmann B, DiMeglio LA, et al. Contrasting the clinical care and outcomes of 2,622 children with type 1 diabetes less than 6 years of age in the United States T1D Exchange and German/Austrian DPV registries. Diabetologia. 2014;57(8):1578–85.

10. Murillo S, Brugnara L, Maduell X, Novials A. Management of Hypoglycemia in Adults with Type 1 Diabetes in Real-Life Condition. Ann Nutr Metab. 2020;76(4):277–84.

11. Singh P, Jain A, Kaur G. Impact of hypoglycemia and diabetes on CNS: Correlation of mitochondrial oxidative stress with DNA damage. Mol Cell Biochem. 2004;260(1):153–9.

12. Joy NG, Hedrington MS, Briscoe VJ, Tate DB, Ertl AC, Davis SN. Effects of acute hypoglycemia on inflammatory and pro-atherothrombotic biomarkers in individuals with type 1 diabetes and healthy individuals. Diabetes Care. 2010;33(7):1529–35.

13. Wang J, Alexanian A, Ying R, Kizhakekuttu TJ, Dharmashankar K, Vasquez-Vivar J, et al. Acute exposure to low glucose rapidly induces endothelial dysfunction and mitochondrial oxidative stress role for AMP kinase. Arterioscler Thromb Vasc Biol. 2012;32(3):712–20.

14. Charlton A, Garzarella J, Jandeleit-Dahm KAM, Jha JC. Oxidative stress and inflammation in renal and cardiovascular complications of diabetes. Biology (Basel). 2021;10(1):1–18.

15. Dos Santos JM, Tewari S, Mendes RH. The role of oxidative stress in the development of diabetes mellitus and its complications. J Diabetes Res. 2019;2019:10–3.

16. Ceriello A, Novials A, Ortega E, La Sala L, Pujadas G, Testa R, et al. Evidence that hyperglycemia after recovery from hypoglycemia worsens endothelial function and increases oxidative stress and inflammation in healthy control subjects and subjects with type 1 diabetes. Diabetes. 2012;61(11):2993–7.

17. Colinet V, Lysy PA. Characterization of Post-Hypoglycemic Hyperglycemia in Children and Adolescents With Type 1 Diabetes: The EPHICA Study. Front Endocrinol (Lausanne). 2022;13:1225.

18. Zhou Z, Sun B, Huang S, Zhu C, Bian M. Glycemic variability: adverse clinical outcomes and how to improve it? Cardiovasc Diabetol. 2020;19(1):1–14.

19. Pollé OG, Delfosse A, Martin M, Louis J, Gies I, den Brinker M, et al. Glycemic Variability Patterns Strongly Correlate With Partial Remission Status in Children With Newly Diagnosed Type 1 Diabetes. Diabetes Care. 2022;45(October):2360–8.

20. Cole TJ, Lobstein T. Extended international (IOTF) body mass index cut-offs for thinness, overweight and obesity. Pediatr Obes. 2012 Aug;7(4):284–94.

21. Watkins W, Taylor M. Calculating areas of irregulars polygons. PRIMUS. 1993 Jan;3(4):379–88.

22. Mortensen HB, Hougaard P, Swift P, Hansen L, Holl RW, Hoey H, et al. New definition for the partial remission period in children and adolescents with type 1 diabetes. Diabetes Care. 2009;32(8):1384–90.

23. Wentworth JM, Bediaga NG, Giles LC, Ehlers M, Gitelman SE, Geyer S, et al. Beta cell function in type 1 diabetes determined from clinical and fasting biochemical variables. Diabetologia. 2019;62(1):33–40.

24. SIMES RJ. An improved Bonferroni procedure for multiple tests of significance. Biometrika. 1986;73(3):751–4.

25. Bates D, Mächler M, Bolker BM, Walker SC. Fitting linear mixed-effects models using lme4. J Stat Softw. 2015;67(1).

26. Kuznetsova A, Brockhoff PB, Christensen RHB. lmerTest Package: Tests in Linear Mixed Effects Models . J Stat Softw. 2017;82(13).

27. Stephenson JM, Schernthaner G. Dawn phenomenon and somogyi effect in IDDM. Diabetes Care. 1989;12(4):245–51.

28. Rickels MR, Evans-Molina C, Bahnson HT, Ylescupidez A, Nadeau KJ,

Hao W, et al. High residual C-peptide likely contributes to glycemic control in type 1 diabetes. J Clin Invest. 2020;130(4):1850–62.

29. Akirav E, Kushner JA, Herold KC. β-Cell Mass and Type 1 Diabetes: Going, Going, Gone? Diabetes. 2008;57(11):2883–8.

30. Greenbaum CJ, Thomas MP, Mcgee PF, Battelino T, Haastert B, Ludvigsson J, et al. Mixed-meal tolerance test versus glucagon stimulation test for the assessment of β-cell function in therapeutic trials in type 1 diabetes. Diabetes Care. 2008 Oct 1;31(10):1966–71.

31. Mørk FCB, Madsen JOB, Jensen AK, Hall G V., Pilgaard KA, Pociot F, et al. Differences in insulin sensitivity in the partial remission phase of childhood type 1 diabetes; a longitudinal cohort study. Diabet Med. 2022 Feb 1;39(2):e14702.

32. Nalysnyk L, Hernandez-Medina M, Krishnarajah G. Glycaemic variability and complications in patients with diabetes mellitus: Evidence from a systematic review of the literature. Diabetes, Obes Metab. 2010;12(4):288–98.

33. Nathan DM, Turgeon H, Regan S. Relationship between glycated haemoglobin levels and mean glucose levels over time. Diabetologia. 2007;50(11):2239–44.

34. Monnier L, Colette C, Wojtusciszyn A, Dejager S, Renard E, Molinari N, et al. Toward defining the threshold between low and high glucose variability in diabetes. Diabetes Care. 2017;40(7):832–8.

35. Martyn-Nemeth P, Quinn L, Penckofer S, Park C, Hofer V, Burke L. Fear of hypoglycemia: Influence on glycemic variability and self-management behavior in young adults with type 1 diabetes. J Diabetes Complications. 2017;31(4):735–41.

3.6 Paper III: Pancreas Imaging of Children with Type 1 Diabetes Reveals New Patterns and Correlations with Pancreatic Functions

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Short running title: Pancreas imaging in pediatric new onset type 1 diabetes

Keywords: Type 1 diabetes, children, pancreas, imaging, exocrine, topography

3.6.1 *Structured abstract*

Context: Type 1 diabetes (T1D) is a heterogeneous disease affecting the islets and the exocrine pancreas. How the topographical distribution of the involved tissue lesions might correlate with patient phenotype and pancreas functions is uncertain.

Objective: To perform a longitudinal multiparametric characterization of the pancreas of children with new-onset T1D and investigate the topographic correlations between magnetic resonance imaging (MRI) indices and pancreatic functions during the first year postdiagnosis.

Methods: Pancreas volume (PV) from 31 children with new-onset T1D was compared to 29 retrospectively identified age-, BMI- and sex-matched controls according to pancreatic subregions (total, head, body and tail) and pubertal status. Associations between MRI indices and markers of pancreatic functions were assessed using uni- and multivariate regression models during the first year postdiagnosis, and during longitudinal follow-up in a subset of patients (n=22).

Results: Pediatric patients with new-onset T1D (48% prepubertal) demonstrated an age-related homogenous reduction of PV at diagnosis compared to controls (-45±7%), with prepubertal patients having increased pancreas atrophy (+25±2.1%). Pancreatic MRI indices were topographically correlated with markers of pancreatic functions (C-peptide, trypsinogen) and improved prediction models of glucose homeostasis. Longitudinal evolution of PV indices at 1-year postdiagnosis were inversely correlated with pancreas atrophy at diagnosis ($R=-0.72$, $p<0.001$) but not with markers of glucose homeostasis.

Conclusion: Thorough longitudinal analysis of pancreas of children with T1D using MRI might improve the understanding of T1D heterogeneity (onset and evolution), support a more aggressive disease in children with young-onset T1D and provide potential additional markers of pancreatic functions.

3.6.2 Introduction

One of the paramount challenges in modern medicine is the clinically relevant characterization of disease heterogeneity between patients, with the perspective of personalization of care. In the quest for precision medicine and disease biomarkers, type 1 diabetes (T1D) is a spearhead as a single nosological entity that gathers a wide array of clinical entities. This situation currently leads to poor integration of the disease patchiness in the clinical routine and major barriers in translational research.

T1D is one of the most prevalent chronic autoimmune diseases during childhood, which is characterized by the progressive destruction of insulin-secreting β cells. However, major questions remain unsolved; especially the nature of immune attack (e.g., autoimmune process or β-cell vulnerability) (1), the extent of disease heterogeneity (in terms of clinical onset and evolution) (2), and the lack of biomarkers able to predict the evolution of residual endocrine function (3–5). The era of omics and multiplexing opened the way to hypothesis-free studies that shed the light on key aspects of T1D pathogeny including interactions between β and acinar cells, renewing the hypothesis of T1D as a whole-pancreas disease (6,7).

Although β cells only represent ~2% of the total pancreatic mass, it is known for more than a century that patients with T1D may present both a reduction of half of their pancreas weight and pancreatic exocrine dysfunction (8,9). Recent studies showed that pancreas atrophy is already present in the earliest stages of T1D, with presymptomatic insulin-autoantibodies (IAb) positive individuals exhibiting intermediate levels of

pancreatic volume (i.e., between new-onset T1D and control patients) (10). Some first-degree relatives of patients with T1D also developed autoantibodies against acinar cells (e.g., anti-BDSL) before progressing with anti-islet autoimmunity and finally overt T1D (11). Finally, up to 70% of patients with T1D had circulating autoantibodies against the exocrine tissue, supporting a global immune attack of the pancreas in a part of the individuals (12–14). Nonetheless, the exact temporality (before, concomitantly, or after β-cell demise) and mechanisms underlying the structural modifications and dysfunction of the exocrine pancreas remain largely unknown. Also, phenotypic translation of exocrine insufficiency is highly disparate among patients with T1D (9,10,15).

Endotypes (i.e., groups of individuals with specific histological, genetic and clinical features) were recently described in patients with new-onset T1D and suggested distinct etiopathological mechanisms (1,16–18). These endotypes showed age-related differences (<7 years-old or >12 years-old) with young patients demonstrating a more aggressive T1D pattern characterized by higher incidence of high-risk genotypes (18), increased markers of inflammation and immunity at T1D onset (19) and a steeper decline of residual β-cell secretion in the weeks following T1D diagnosis (20). While histological distinctions between endotypes mostly relied on endocrine compartment (e.g., immune islet infiltration and insulin processing) (16,17), differences have not yet been investigated for exocrine features.

Magnetic Resonance Imaging (MRI) is a cardinal tool in the evaluation of pancreatic pathologies as it allows real-time and *in situ* quantification of multiple tissue parameters (e.g., volume, structural modifications, tissue perfusion and diffusion, fat disposal) from the age of 5 years old (21). Corroborating previous anatomical and histological descriptions, MRI of patients with T1D showed a reduction of pancreas volume, alterations of pancreas microvasculature and a patchy macrophage infiltration of the whole organ (15,22). MRI also proved to be minimally invasive, cost-effective, and repeatable in the context of longitudinal pediatric studies (21,23). Few MRI studies investigated pediatric T1D cohorts and were often focused on the evaluation of a single MRI parameter, the pancreatic volume (PV). However, no clear trend appeared in the correlation between these imaging features, and both exocrine and endocrine functions (10,15,24–28). The incomplete characterization of patient phenotype (in terms of cross-sectional measurements of both endocrine and exocrine functions) and the lack of topographic measurements of pancreatic indices may explain this discrepancy between studies.

The purpose of this subsidiary study of the DIATAG cohort is to perform a topographic and phenotypic characterization of the pancreas in pediatric patients with new-onset T1D. Uni- and multiple correlations between pancreas MRI indices, and exocrine and endocrine pancreatic functions were thus assessed upon diagnosis (baseline study) and after 12 months (longitudinal study).

3.6.3 Material and methods

Study design

This study included a subset of participants from the Belgian multicentric DIATAG cohort. The aim of DIATAG trial was to characterize the heterogeneity of β-cell function evolution in pediatric patients with new-onset T1D during the first year after clinical-onset. Inclusion and exclusion criteria were previously described (29). T1D was diagnosed using ISPAD criteria (30) which included at least one circulating anti-islet autoantibody (anti-insulin, anti-protein tyrosine phosphatase, anti-glutamic acid decarboxylase or, anti-Zinc transporter 8). All participants and their parents gave their written consent prior to the enrolment in the study. Exclusion and inclusion criteria are provided elsewhere (29). The protocol was approved by the principal ethical committee (Comité d'Ethique Hospitalo-Facultaire of Cliniques universitaires Saint-Luc (CUSL), [2018/04DEC/462]) and local ethical committees of all participating institutions. The trial was registered at www.clinicaltrial.gov (NCT04007809).

Study procedures

The baseline screening included the patient demographics at diagnosis and a blood draw that was performed after an overnight fast between +5 to +21 days. Pubertal status was determined during the initial hospitalisation either by pediatric endocrinologists (Tanner stage) or serum LH levels when clinical examination was borderline (e.g., early M2 or testicular volume 4-6mL) or absent. Participants were either classified as

prepubertal (Tanner I or LH<0.3 UI/L) or pubertal (Tanner II-V or LH>0.3 UI/L). After the initial hospitalization, the outpatient clinical follow-up in diabetes care conventions was organized throughout routine visits at +3, +6, +9 and +12 months as previously described (29). During these visits, residual β-cell secretion and routine parameters (i.e., total insulin daily dose [TDD], $IDAA_{1C}$, HbA_{1C}) were evaluated. Data from patients' medical records were gathered and registered inside the Research Electronic Data Capture (REDCap) system (31).

Measure of residual β-cell secretion

Sample collection and assay characteristics were previously described (29). Estimated residual stimulated C-peptide ($CPEP_{EST}$) was evaluated at +3 months and +12 months using a mathematical formula that integrates both biological measures before stimulation test (i.e., $CPEP_{BASAL}$ [fasting C-peptide], fasting plasma glucose, HbA_{1C}) and clinical parameters (i.e., BMI, TDD, disease duration) (32). In this study, we used $CPEP_{EST}$ as data were available for most patients and demonstrated strong correlations with stimulated C-peptide with both glucagon stimulation tests (29) and mixed-meal tolerance test (33).

Remission status

Remission status was determined using the insulin dose-adjusted $A1_C$ ($IDAA_{1C}$) score, as follows: HbA1c (%) + (4 × insulin dose (U/kg body weight per 24 h) (34), with a score below 9 defining the occurrence of remission. TDD was either reported by patients (MDI users) or calculated using the software for pump users.

Pancreatic exocrine function

The exocrine function was evaluated by measuring serum immunoreactive trypsinogen levels on fasting samples at baseline. Serum immunoreactive trypsinogen levels were determined using radioimmunoassay (KIPCE07, DIAsource ImmunoAssays S.A., Louvain-la-Neuve, Belgium) at CUSL central laboratory, with a normal laboratory reference range from 140 to 400 ng/mL and inter- and intra-assay coefficients of variation respectively from 4.9% to 6.7% and 2.8% to 3.3%.

Multiparametric pancreas MRI assessment

Participants aged more than five years (<100 days after diagnosis) underwent a pancreas MR imaging without injection or sedation at CUSL. Participants were not fasted before imaging (15,21). Clinical metrics (weight in kg and height in cm) were collected on the day of the MRI scan or from a recent medical visit (< 1 month, > 30 days after diagnosis). MRI was repeated for a subset of participants after a minimum of 10 months to allow the longitudinal assessment of quantitative parameters (see below).

Pancreas imaging was performed on a 3T Signa Premier MRI scanner (GE Healthcare, Milwaukee, WI, USA). Axial MR images were acquired from inferior to superior, until the whole pancreas volume (PV) was covered. Parameters of the MRI sequences are given in **Supplementary Table 1**.

Anatomical imaging & segmentation of pancreas

A 2D multislice T2-weighted Single-Shot Fast Spin Echo (T2w-SSFSE) sequence and a 3D T1-weighted SPoiled GRadient (T1w-SPGR, LAVA Flex) echo sequence were used for anatomical characterization. Pancreas volume (PV) measures were performed independently by O.P., A.D. and P.C. Pancreas subregions segmentation was performed by O.P. and A.D.. The pancreas was manually delineated on each imaging slice from the water-only series of the T1w-SPGR sequence using an off-line image processing workstation (Vitrea FX, Vital Images, Minnetonka, MN, USA) which generated automatically the volume (mL). T2w-SSFSE images were used when the pancreas could not be accurately delineated on T1w-SPGR images. When inter-observer differences between PV measures exceeded 10%, images were reviewed in consensus. The pancreas index (PI) was calculated by dividing the PV by the weight of the participant during the first outpatient visit (i.e., baseline study) (10) or at 12-month visit (i.e., longitudinal study).

Pancreas subregions were defined as follows: head, body and tail. The head was defined as the organ subregion located from the duodenum to the left border of the superior mesenteric vein. The body was defined as the subregion downstream of the pancreas head to the median border of the left kidney. The tail was defined as the subregion that lies anteriorly to the left kidney, from its median border to the splenic hilum (**Supplementary Fig. 1**) (35–37). A pancreas volume ratio

(PVR) was computed to investigate the topography of pancreas atrophy and calculated as follows:

$$PVR = \frac{\mathrm{PV}\ of\ the\ tail\ + PV\ of\ the\ body}{PV\ of\ the\ head}$$

To classify patients with T1D according to PV indices at 12 months, PI ratio was calculated as PI^{T12}/PI^{T0}. To evaluate the correlations between longitudinal evolution of MRI indices and clinical parameters during the first year of T1D, absolute and relative differences were respectively calculated as follows: $Parameter^{T0}$ - $Parameter^{T12}$ and ($Parameter^{T0}$ - $Parameter^{T12}$)/ $Parameter^{T0}$.

Controls were retrospectively identified using the Picture Archiving and Communication Systems database in the participating centers (CUSL, Grand hôpital de Charleroi and CHC MontLégia) and matched with diabetic participants for age, sex and BMI. Medical records were screened by a board-certified attending paediatrician (OP) to exclude patients with history of pancreatic disease (either pancreatic masses, pancreatic trauma, medical history of pancreatitis, ciliopathy or, cystic fibrosis) and/or treatment with pancreas toxicity (radiotherapy, chemotherapy) (38). MRI scans with incomplete PV or blurred pancreatic outlines were excluded from the analysis. Volume measurements were performed identically to the T1D cohort. The reading physician was not blinded to the patient condition in order to identify possible cofounding images.

Diffusion Weighted Imaging (DWI)

DWI was performed with a single-shot Echo Planar Imaging (EPI) sequence with diffusion encoding applied in three orthogonal directions for three b-values (0, 50 and 800 s/mm²).In order to minimize the contribution of the microperfusion, only the two largest b-values were used to compute the ADC (Apparent Diffusion Coefficient).

Fat quantification

A 3D Multi-echo GRadient Echo (Multi-echo GRE, IDEAL-IQ) sequence with a 3° flip angle was used for fat quantification. Six equidistant echoes were acquired (echo times TE from 0.9 ms to 4.3 ms) to reconstruct the in-phase, out-of-phase, water (W), fat (F) and R2* = 1/T2* maps with the manufacturer's algorithm IDEAL-IQ. All ADC maps and Fat-Fraction (FF, F/(F+W)) maps were computed offline by MRI physicists using in-house software under Matlab (Matlab R2011b, MathWorks, Natick, MA, USA).

Statistical analysis

Data analysis was performed using Matlab (R2021b Update 2, Mathworks) and R (R Core Team) software. A p-value <0.05 was regarded as statistically significant for all analyses.

Demographic and clinical data were reported as the mean ± SD for continuous variables and as numbers and proportions for categorical variables. Differences in group means were assessed using a Student t-test (equivalently a Mann-Whitney U-test when data distributions were non normal). Differences in proportions were assessed using chi-square or Fisher exact test as appropriate. . A paired t-test (equivalently a

Wilcoxon test) was used when assessing mean differences over time within a same group (longitudinal study). A 4-way ANOVA with fixed effects was performed to assess the impact of disease status, sex, pubertal stage and anatomical subregions on MRI indices (PV, PI and PVR).

Correlations between parameters of interest were assessed measuring the Pearson's correlation coefficient (equivalently the Spearman's correlation coefficient). Strength of correlation was interpreted as follows: $R < 0.40$ = weak, $0.41 \leq R < 0.60$ = fair, $0.6 \leq R < 0.80$ = moderate, and $R \geq 0.81$ = strong (39).

Multivariate linear regression models were used to assess whether MRI indices (whole pancreas, head, body and tail subregions) at diagnosis predicted endocrine functions of the patient at +3, +6, +9, +12 months postdiagnosis. A backward procedure to enter the parameters in the model (with an associated significance level of $p<0.20$ to be entered and of $p>0.04$ to be removed) was chosen. The multiple correlation coefficient R of each model was reported.

3.6.4 Results

Demographics

The study included thirty-one pediatric patients (59% male) with new-onset T1D from the DIATAG trial and twenty-nine retrospectively identified non-diabetic controls matched for age, sex, BMI, and pubertal status. Patients with new-onset T1D underwent an initial MRI scan at a mean ± SD age of 11.2 ± 2.9 years, shortly after clinical diagnosis (mean duration = 40 ± 37

days) (**Table 1**). Graphical representation of study design is provided in **Fig. 1** (**H1 to H4**)**.**

Table 1: Population characteristics

	Global (N=60)	*Controls (N = 29)*	*T1D (N = 31)*	*P-value**
Distribution				
Age — years[#]	11.4 ± 3.1	11.6 ± 3.3	11.2 ± 2.9	0.70[†]
Height (Z-score)	-0.15 ± 1.12	-0.37 ± 1.05	0.04 ± 1.12	0.22[†]
Weight (Z-score)	-0.16 ± 1.01	-0.32 ± 0.91	0.00 ± 1.05	0.24[†]
BMI (Z-score)	0.02 ± 1.12	-0.14 ± 1.12	0.12 ± 1.08	0.36[†]
Sex — Female no. (%)	25 (41.7)	12 (41)	13 (43)	>0.99[‡]
Prepuber — no. (%)	29 (48.3)	15 (51.7)	14 (45.1)	>0.99[‡]
Baseline diabetes characteristics				
HbA_{1C} — %	NA	NA	12.8 ± 2.0	NA
Presence of ketoacidosis — no. (%)	NA	NA	9 (29)	NA
Glycaemia — mg/dL	NA	NA	540 ± 196	NA
Weight loss — %	NA	NA	13.5 ± 8.2	NA
Diabetes duration at imaging — days	NA	NA	40 ± 37	NA
Pancreas volume indices				
PV — mL	33 ± 15	43.1 ± 12.7	23.4 ± 10.1	<0.001[†]
Pancreatic index — mL/kg*	0.9 ± 0.4	1.15 ± 0.3	0.6 ± 0.2	<0.001[†]
Pancreas volume ratio	1.4 ± 0.6	1.5 ± 0.4	1.4 ± 0.7	0.74[†]
Glycemic control[||]				
Remitters — no. (%)	NA	NA	20 (66)	NA
HbA_{1C} — %	NA	NA	6.0 ± 0.7	NA
Insulin doses (IU/kg/day)	NA	NA	0.6 ± 0.3	NA
$IDAA_{1C}$	NA	NA	8.5 ± 1.4	NA
$CPEP_{EST}$ [§]	NA	NA	0.5 ± 0.3	NA
Exocrine function				
Trypsinogen (ng/mL)	NA	NA	234 ± 85	NA

Legend: Plus–minus values are means ±SD. Percentages may not total to 100 because of rounding. *p-value calculated between type 1 diabetes and control group. results were considered as significant when under 0.05. † Student t-test, ‡ Chi-square, || Parameters evaluated at +3 months after diagnosis, ¶ Wilcoxon-test § calculated as described by Wentworth et al. Abbreviations: BMI = Body Mass Index, HbA1C = Glycated hemoglobin level, IDAA1C = insulin dose-adjusted A1C, NA = not applicable, T1D = type 1 diabetes.

Pancreas volume is decreased in patients with a new diagnosis of T1D (H1)

We first investigated whether patients with T1D presented pancreas atrophy at the time of clinical onset (**Fig. 1, H1**). Because PV was dependent on body weight ($p < 0.050$ at ANOVA), most analyses were based on PI as previously described (9,15,22,38).

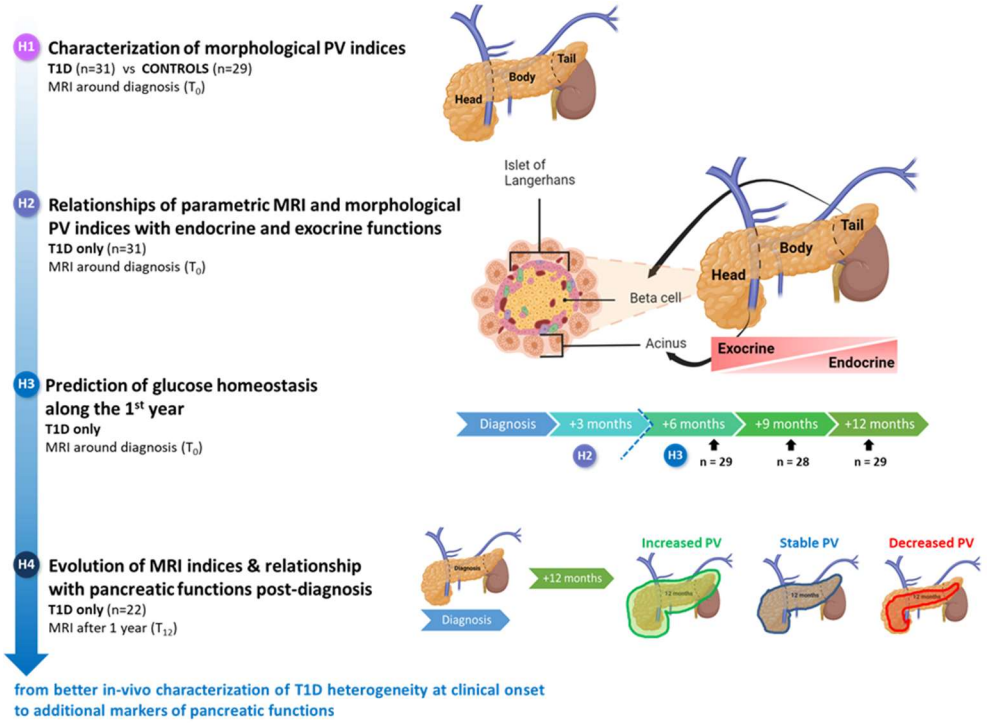

Figure 1: Flowchart of the DIATAG MRI study. Graphical representation of main hypothesis (H1-H4) investigated in the study. H1: analysis performed on anatomical MRI imaging (i.e., PV, PI and PVR) according to the disease status, the pancreas subregions and the pubertal status (T_0). H2: analysis performed on anatomical, diffusion and FAT MRI (T_0). Exocrine (i.e., trypsine) and endocrine ($CPEP_{EST}$, IDAA1C, HbA1C and TDD) functions were evaluated around diagnosis. H3: analysis performed on anatomical, diffusion and FAT MRI (T_0). Exocrine (i.e., trypsine) and endocrine ($CPEP_{EST}$, IDAA1C, HbA1C and TDD) were evaluated at +12 months, and +6, +9, +12 months respectively. H4: analysis performed on anatomical, diffusion and FAT MRI (T_{12}). Exocrine (i.e., trypsine) and endocrine ($CPEP_{EST}$, $IDAA_{1C}$, HbA_{1C} and TDD) were evaluated at +12 months. Abbreviations : H, hypothesis; MRI, magnetic resonance imaging; PV, pancreatic volume; PI, pancreatic index; PVR, pancreas volume ratio.

As shown in **Fig. 2A-B**, PV and PI were significantly decreased in patients with new-onset T1D when compared to their matched non-diabetic controls, with a mean atrophy of respectively -45% ± 7.2% and -49% ± 6.0% ($p < 0.001$) (**Table 1**). PV was substantially correlated with age in both T1D and control groups ($R = 0.61$ and $R = 0.71$ respectively, $p < 0.001$). While PI was negatively correlated with age in controls ($R = -0.41$, $p = 0.032$), no correlation was observed in patients with T1D ($p > 0.050$) with amplitude of PI differences (between non-diabetic control and T1D) being more important in children with young onset T1D (**Fig. 2C**).

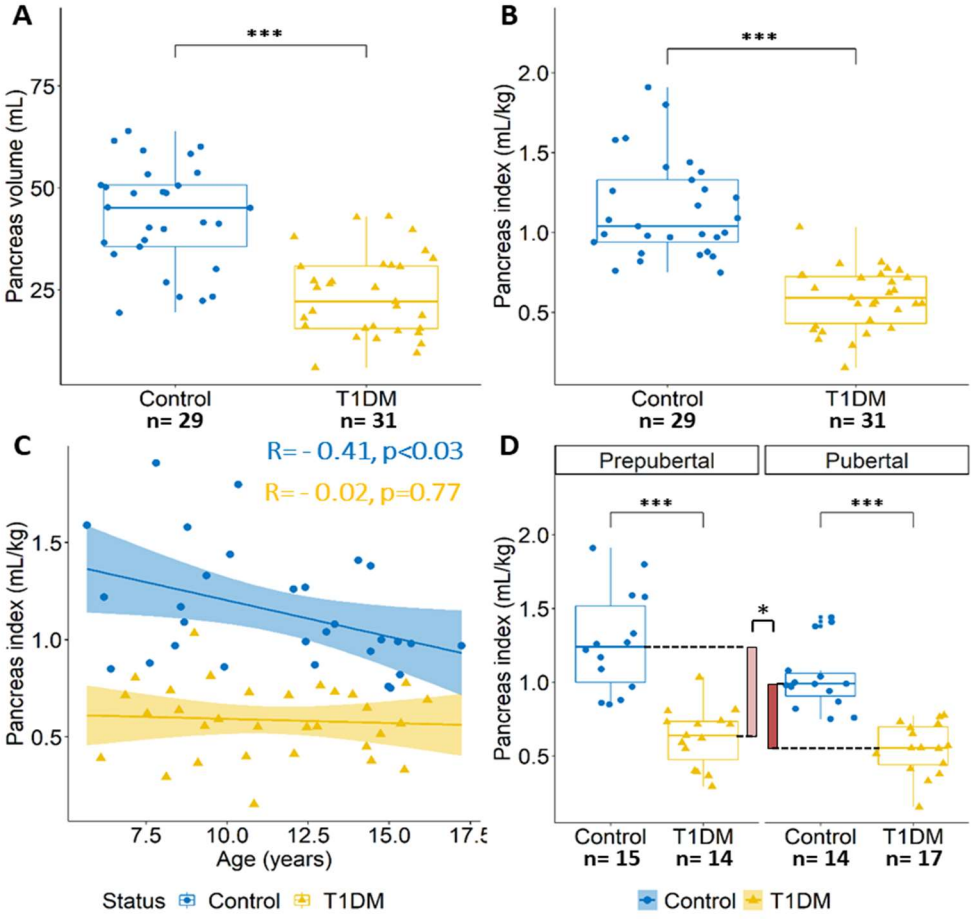

Figure 2: Pancreas volume indices according to age and pubertal status in new-onset T1DM patients and age-, sex-, BMI-matched controls. MRI was performed closed to the diagnosis of T1D. Panels A-B represent pancreas volume (**A**) and pancreas index (**B**) in T1DM patients (yellow) and controls (blue). Panel **C** represents the evolution of pancreas index according to the age of the patient and the disease group (yellow = T1DM, blue = controls). Shaded zone around regression lines represent 95% confidence interval. Panel **D** represents the influence of pubertal status on pancreas atrophy between T1DM (yellow) and controls (blue). Vertical bars represents differences of median between prepubertal (light orange) and pubertal (dark orange) patients. Box plots display the median, 25th and 75th percentiles. The significance level is represented either by numerical values or signs (*=p<0.05,***= p<0.0001). Abbreviations : MRI, magnetic resonance imaging; R, spearman rho; T1DM, Type 1 diabetes mellitus.

The highest levels of pancreas atrophy were observed in patients with prepubertal onset of T1D (H1)

To deepen the characterization of pancreas atrophy in patients with T1D, we looked at whether pancreas atrophy **a)** varied according to the pubertal status and **b)** showed specific topographic patterns (diffuse or focal lesions) (**Fig. 1, H1**).

PVR was not influenced by the disease status and the pubertal stage while PV and PI depended on both factors and the anatomical area ($p < 0.001$). All three indices were not significantly dependent of sex. Volume indices (i.e., PV and PI) were significantly reduced in the T1D group regardless of the anatomical area and pubertal status (**Supplementary Table 2**). Analysis of these data according to pubertal status showed specific patterns for PI but not PV in our patients. Patients with prepubertal onset of T1D had a greater reduction of PI compared to pubertal patients (PI difference of mean ± SEM, 0.65 mL/kg ± 0.09 mL/kg *vs* 0.49 mL/kg ± 0.05 mL/kg; $p < 0.001$) corresponding to a decrease of -25% ± 2.1% in prepubertal T1D group (**Fig. 2D**). Investigating the topography of pancreas destruction, PVR was similar in both disease groups ($PVR^{T1D} = 1.4 \pm 0.7$, $PVR^{CONTROL} = 1.5 \pm 0.4$, $p = 0.743$) independently of the pubertal status.

At T1D onset, PV indices correlated with pancreatic functions but not with clinical phenotype (H2)

Our next approach was to investigate whether pancreas atrophy at clinical onset was associated with diabetes severity and pancreatic exocrine dysfunction. Correlations between pancreatic MRI measures and

parameters at +3 months for endocrine function, and at diagnosis for exocrine function test were assessed **(Fig. 1, H2).** As pancreatic cell populations heterotopically distribute across the pancreas (e.g., most islets being located in pancreatic tail) (40,41), correlations were assessed in both the whole pancreas and pancreatic subregions (head [$Parameter_{HEAD}$], body [$Parameter_{BODY}$] and tail [$Parameter_{TAIL}$]) (**Supplementray Table 3**).

Baseline characteristics of diabetes (i.e., HbA_{1C}, C-peptide, bicarbonates, blood neutrophils absolute count, glycemia) were not correlated with PV indices or MRI parametric values (all $p > 0.050$, *data not shown*). Accordingly, patients presenting an inaugural episode of DKA (n = 9) did not show any difference in PV indices compared to patients without DKA (n = 22) (*data not shown*). These correlations did not differ when classifying volumes according to pancreatic subregions (all $p > 0.050$, *data not shown*).

Correlations between PV indices and additional parameters like residual β-cell secretion (i.e., $CPEP_{EST}$ or $CPEP_{BASAL}$, n = 29) or glycemic control (i.e., clinical parameters, n = 31) measured close to diagnosis were also evaluated. While $CPEP_{EST}$ and $CPEP_{BASAL}$ were not correlated with PV and PI of the whole pancreas (all $p > 0.050$, *data not shown*), both residual secretion estimates showed moderate correlations with pancreatic tail volume (PV_{TAIL}; respectively $R = 0.55$, $p = 0.002$; $R = 0.50$, $p < 0.009$) (**Fig. 3A**) but not with pancreatic head volume (PV_{HEAD}) or pancreatic body volume (PV_{BODY}). No correlation was found between clinical parameters at +3 months ($IDAA_{1C}$, IDD or, HbA_{1C}) and PV indices independently of pancreatic subregions (all $p > 0.050$, *data not shown*).

As pancreas mass is mostly composed of acinar cells, we investigated whether circulating markers of exocrine function (fasting serum lipase and trypsinogen, n=31) measured around MRI scan witnessed the pancreas atrophy in patients with T1D. Most of the trypsinogen values were within the laboratory ranges (n=28 [90%]). Serum trypsinogen and lipase were correlated with both PV and PI of the whole pancreas with the strongest correlations observed for PI (respectively $R = 0.52$, $R = 0.58$, $p < 0.003$) (**Supplementary Figure 2, Supplementary Table 3**). Topographical pancreatic analysis showed a specific correlation between trypsinogen and PV_{HEAD} ($R=0.49$, $p=0.006$) (**Fig. 3B**) but not PV_{TAIL} or PV_{BODY}.

While PV indices of the whole pancreas were solely correlated with markers of exocrine function, topographical subanalysis revealed correlations between pancreatic regions subvolumes and both markers of exocrine and endocrine function around T1D diagnosis.

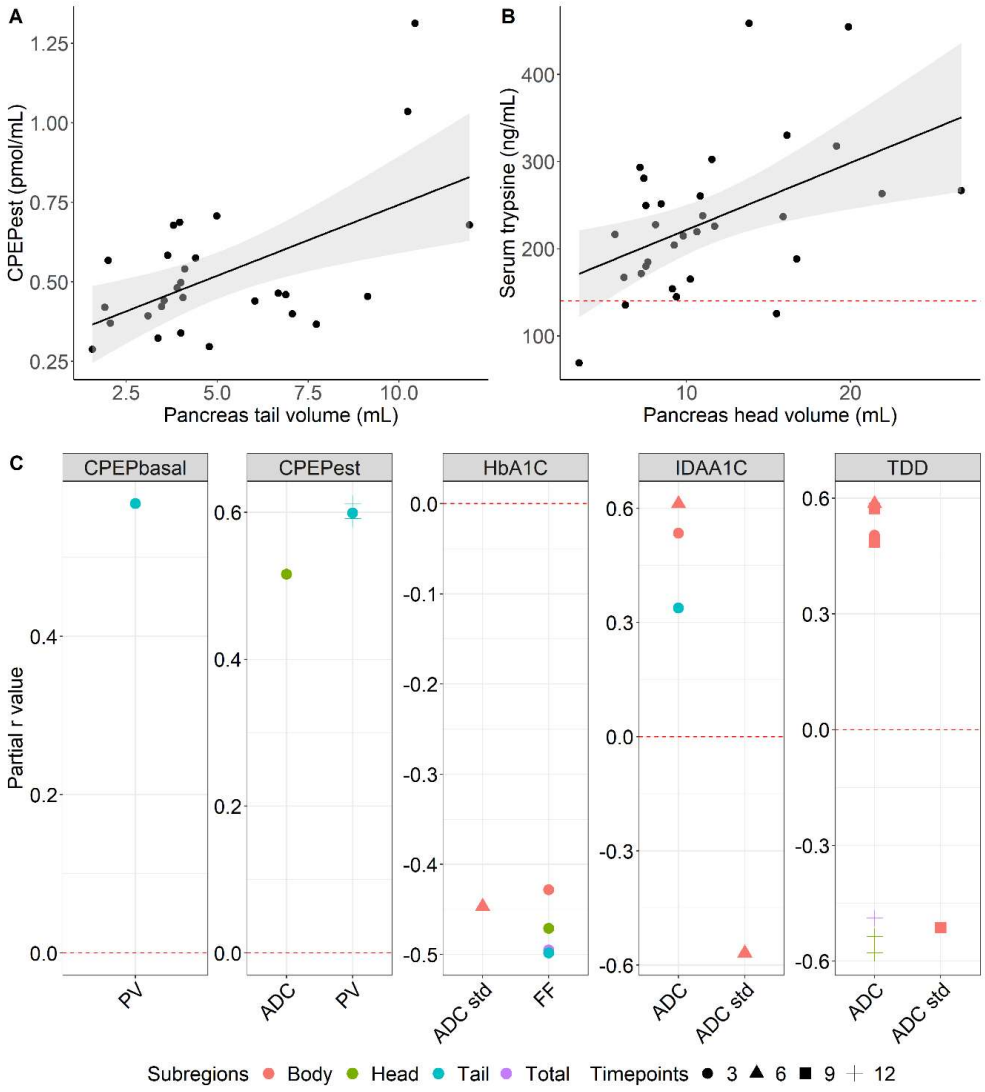

Figure 3: Pancreas MRI parameters at diagnosis correlate with residual pancreatic functions and improve prediction models of glucose homeostasis during the 1^{rst} year of T1D. Statistically significant topographic correlations between regional pancreatic subvolumes and residual B-cell secretion (**A**) and exocrine function (**B**) with shaded zone around regression lines represent 95% confidence interval. The horizontal dashed red line in panel **B** represents the lower range of laboratory normality threshold. (**C**) Graphical representation of partial r values in multivariate prediction models including MRI parameters (x axis). The horizontal dashed red line

corresponds to R=0. Dots are colored according to pancreatic subregions (PV_{BODY} = red, PV_{HEAD} = green, PV_{TAIL} = blue, PV_{TOTAL} = purple) and shaped according to time from diagnosis (+6 months = round, +9 months = triangle, +12 months = square). The significance level is 0.05. Abbreviations: ADC = Apparent diffusion coefficient; FF= Fat fraction; PI = pancreas index; PV = pancreas volume; R= Spearman rho; StADC = standard deviation of ADC; StFF = standard deviation of FF; T1D = type 1 diabetes.

Pancreas volume indices predicted the extent of exocrine function at diagnosis and improved prediction models of endocrine function during the first year after T1D onset (H3)

We next studied whether volume indices and parametric MRI values at diagnosis predicted pancreatic functions around clinical onset and during the first year of T1D using mono- and multivariate models (**Fig. 1, H2-H3**). Clinical parameters (HbA_{1C}, $IDAA_{1C}$, IDD) were collected at Δ+3 months (n = 31), Δ+6 months (n = 29), Δ+9 months (n = 28) and Δ+12 months (n = 29). Residual β-cell secretion estimates ($CPEP_{EST}$ and $CPEP_{BASAL}$) were collected at Δ+3 months (n = 29) and Δ+12 months (n = 25).

Around the diagnosis **(Fig. 1, H2)**, monoparametric models based on whole PV and PI predicted serum trypsinogen at diagnosis (R = 0.49 and R = 0.52 respectively, $p < 0.009$), but not residual β-cell secretion or glucose homeostasis parameters at Δ+3 months (all $p > 0.050$, *data not shown*). A biparametric model including whole pancreas FF and pubertal status predicted HbA_{1C} levels at Δ+3 months (R = 0.65, $p < 0.001$) (**Supplementary Table 4**). In pancreatic subregion analysis, PV_{HEAD} moderately predicted serum trypsinogen (R = 0.56, p = 0.002). Two biparametric models including

PV_{TAIL} and sex substantially predicted $CPEP_{EST}$ (R = 0.65, p = 0.002) and $CPEP_{BASAL}$ (R = 0.69, p < 0.001) at Δ+3 months **(Supplementary Table 4)**.

During the first year of T1D **(Fig. 1, H3)**, no individual monoparametric and only two biparametric models including whole pancreas MRI indices predicted markers of glucose homeostasis at Δ+6, +9, +12 months (**Fig. 3C**). However, the inclusion of topographic MRI parameters allowed the identification of several new biparametric models predicting HbA_{1C}, $IDAA_{1C}$, TDD, and $CPEP_{EST}$ during the first year of T1D (**Fig. 3C, Supplementary Table 4**). For example, biparametric models including serum trypsinogen and mean ADC_{BODY} moderately-to-substantially predicted both $IDAA_{1C}$ at Δ+3 months, and TDD at Δ+3, +6, +9 months with a R of 0.64, 0.62, 0.67 and 0.59 respectively (p < 0.005) (**Fig. 3C**). Another biparametric model (puberty status and FF_{TAIL}) showed that being prepubertal and having lower pancreatic FF substantially predicted higher HbA_{1C} at Δ+3 months (R = 0.71, p < 0.001).

Longitudinal evolution of PI was highly heterogeneous among patients and did not reflect the evolution of glucose homeostasis. (H4)

Longitudinal study of pancreas volume indices (PV^{T12} and PI^{T12}) and parametric MRI values after T1D onset was performed. Twenty-two patients (73%) underwent a second imaging session after a mean duration of 14 ± 2.6 months. Pancreatic volumes were normalized for 12 months considering a linear decline or increase (PV^{T12} and PI^{T12}) **(Fig. 1, H4)**.

PV^{T12} was not significantly different from PV measured at diagnosis (PV^{T0}; p > 0.050) (**Fig. 4A**). PI^{T12} was significantly lower at the follow-up MRI compared to PI_{TO} (0.49 mL/kg ± 0.15 mL/kg *vs* 0.58 mL/kg ± 0.19 mL/kg, p = 0.030) with an estimated decline of -0.09 mL/kg ± 0.16 mL/kg during the first year after diagnosis (**Fig. 4A**). This decrease of PI^{T12} was observed in both pancreatic head and body but not in pancreatic tail (p > 0.050) (peak reduction of -0.07 mL/kg ± 0.11 mL/kg in the pancreas body). A large variability was observed in the longitudinal evolution of PI (PI^{T12}/PI^{T0}): respectively 4 (18%), 5 (23%), and 13 (59%) patients increased (PI > 1.05), stabilized (0.95 < PI < 1.05) or decreased (PI < 0.95) their PI during the first year following T1D onset (**Fig. 4B**). For each patient, the trends of PI evolution were globally homogenous within pancreatic subregions (**Fig. 4C**). When we investigated the phenotypic characteristics of these three groups, we found that patients differed by their PI at first MRI scan (PI^{T0}, p = 0.028) but had similar clinical characteristics at diagnosis (either in HbA_{1C}, $HCO3^-$, C-peptide, neutrophils count, plasmatic glycemia or in pubertal status) and during the first year longitudinal follow-up (either inHbA_{1C}, $IDAA_{1C}$, TDD or in serum lipase at Δ+3, +6, +9, +12) (p > 0.050, *data not shown*). The level of PI atrophy (PI^{T12}/PI^{T0}) was indeed inversely correlated with PI^{T0} (R = -0.72, p < 0.001) (**Fig. 4D**). The analysis of parametric MRI maps showed a small decrease in whole pancreatic diffusion at +12 months (ADC^{TO-T12} = -110 mm2/s ± 52.0 mm2/s, p = 0.051) but no significant changes in pancreatic fat fraction or perfusion between both time points.

As structural modifications of pancreas might affect acinar and β cells, we investigated whether differences observed in parameters

measured by MRI (diffusion, perfusion and fat fraction or volume indices) between T_0 and T_{12} witnessed the change of pancreatic functions across the first year **(Fig. 1, H4)**. No significant correlation was found between the absolute or relative differences of volume indices and parameters of glucose homeostasis ($CPEP_{EST}$, HbA_{1C}, $IDAA_{1C}$ or TDD). When studying the parametric maps, differences in diffusion, perfusion and fat fraction were not correlated with differences in endocrine or exocrine function parameters. Those results were independent of the pancreatic subregions.

Finally, we investigated whether topographical correlations residual β-cell secretion ($CPEP_{EST}$, n=21), and volume indices of pancreatic subregions observed around diagnosis persisted at Δ+12 months. PV_{TAIL} remained correlated with $CPEP_{EST}$ measured at +12 months ($R = 0.57$, $p = 0.008$) but not with clinical parameters of glucose homeostasis ($IDAA_{1C}$, TDD or HbA_{1C}; $p > 0.050$) (**Supplementary Figure 3**).

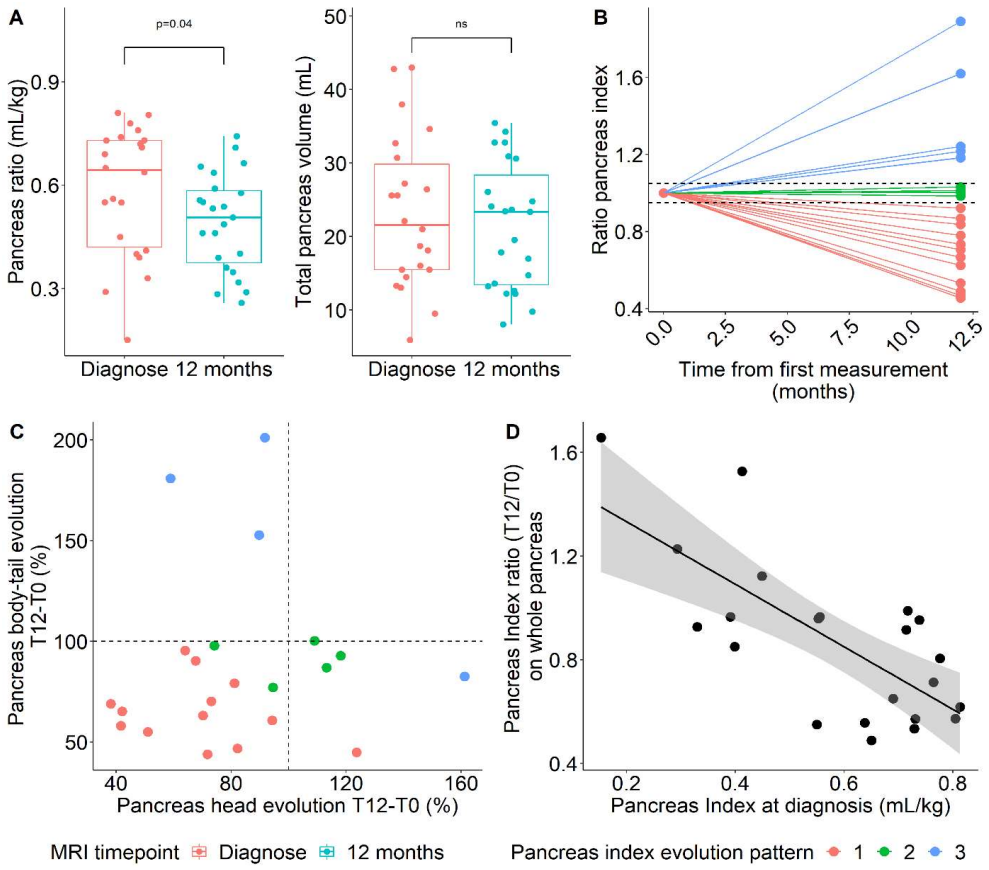

Figure 4: Morphological evolution of pancreas during the first year after T1D onset. (A) Differences in pancreas index (left panel) and pancreas volume (right panel) in T1D patients. **(B)** Visualization of relative evolution of PI during the first year of T1D (PI^{T12}/PI^{T0}) with PI_{T12} being normalized at +12 months considering a linear evolution. Horizontal dashed lines represent pancreas index ratio values of 1.05 (upper) and 0.95 (lower) delineating PI evolution pattern (i.e., increasing (blue dots), stabilizing (green dots) or decreasing (red dots)). **(C)** Topographic visualization of pancreas volume evolution according to body-tail (y axis) and head (x axis) subregions. Colors of the dots represent the PI evolution pattern (i.e., PI^{T12}/PI^{T0}). Dashed lines represent a ratio of 100% (i.e., no change) with value below demonstrating a decrease of PV during the first year of T1D. **(D)** Linear regression with 95% CI bands (shaded zone) between PI at diagnosis and PI ratio (T^{12}/T^{0}). R represent the regression coefficient of Spearman. Significance level was p value <0.05 for all analysis. Abbreviations : CI, confidence interval; PI, pancreas index; T1D, type 1 diabetes.

3.6.5 Discussion and conclusion

The present study showed that the pancreas of pediatric patients with new-onset had homogeneous atrophy across all pancreatic sub-regions with a puberty-related pattern. Indeed, children with prepubertal T1D onset had increased pancreas atrophy compared to the pubertal group (**H1**). Topographic correlations were observed between the volumes of pancreas subregions (PV_{TAIL} and PV_{HEAD}), and residual β-cell secretion and exocrine function respectively measured around T1D diagnosis (**H2**). Topographical correlation with residual β-cell secretion persisted at +12 months. Also, the integration of MRI-related indices measured at diagnosis (e.g., PV indices, ADC, or FF) into biparametric models improved the prediction of glucose homeostasis during the first year after diagnosis (**H3**). Finally, the longitudinal evolution of PI after T1D diagnosis diverged between patients, either increasing or decreasing, and was substantially correlated with PI at diagnosis ($R = -0.72$, $p < 0.001$) but not with routinely assessed parameters at diagnosis or during the follow-up (**H4**).

It remains highly debated as to whether the pancreas atrophy and subclinical exocrine dysfunction result from a primary event involving the whole pancreas or from the primary loss of β-cells (due to loss of insulin trophic factor) (9,42–47). Our data support the first hypothesis, showing a homogeneous distribution of both pancreas atrophy and parametric MRI values across all pancreatic subregions. Our results agreed with *in vivo* and histological findings from previous reports. Imaging studies of T1D pancreas showed similar right and left side pancreatic atrophy (38) with homogeneous distribution of pancreas inflammation in most new-onset T1D

patients (n=6/11) (22). Histological evaluation of pancreases from patients with T1D corroborated these findings as most cell populations from both innate and adaptative immunity showed diffuse tissue infiltration, independently of endocrine or exocrine compartments (42,48,49). These results together support that pancreas atrophy in patients with T1D most likely results from a phenomenon involving the whole organ rather than a specific pancreatic subregion. Nonetheless, our team and others observed a high variability in the level of atrophy among patients with new-onset T1D (15,22,50,51).

Over the past years, there has been compelling evidence that patients with T1D presented both clinical and histological heterogeneity (2,16–19,52,53). Our results showed a puberty-related pattern characterized by increased pancreas atrophy in children with prepubertal T1D onset. Supporting age-related disparities, distinct pancreatic histological patterns (in the endocrine compartment) and specific clinical features were observed in children with young-onset T1D (i.e., < 7 years-old) compared to older-onset (> 12 years-old). These were recently regrouped under the term “endotypes” (2,17–20,52). In that regard, our findings give new insights into age-related differences in pancreas morphology and support a more aggressive disease in children with young T1D-onset (2,17).

Regarding the heterogeneity of pancreas evolution within T1D patients, three patterns in the progression of PV indices during the first year after clinical onset were identified. These patterns hinged only on PI measured at diagnosis and were not correlated with the longitudinal

evolution of clinical routine parameters of glucose homeostasis. Our results suggested that patients with the highest PI at diagnosis had a higher reduction of their PI after onset. These results were also consistent with a recent study that demonstrated a global reduction of PI during the first year after T1D onset (-0.0084 mL/kg/month) with high interpatient variability in PI trajectories (15). These observations together suggest that the evolution of PV indices might indicate a heterogeneous phenomenon starting before T1D onset and continuing during the first year of T1D that is not captured by current markers of diabetes follow-up such as glucose homeostasis (9,51).

This study also showed that indices of whole PV were correlated with markers of exocrine (serum lipase and trypsinogen) but not endocrine function. Similar results were reported by most studies for exocrine function markers (24,28,54). More discordant results were observed for endocrine markers (e.g., residual β-cell secretion, TDD, HbA_{1C}) (10,15,25,26,54,55), probably reflecting the disparities in both evaluation methods and T1D population.

This study revealed moderate topographic correlations between both endocrine and exocrine functions, and pancreatic subvolumes (PV_{TAIL} and, PV_{HEAD} and PI_{HEAD} respectively) at diagnosis and +12 months. Endocrine pancreatic cells (e.g., islets number and β-cell area) distribute heterotopically across the pancreas, being mostly located in the pancreas body and tail in healthy patients (40,41). Supporting these histological descriptions, the incidence of new-onset diabetes mellitus was higher following a distal pancreatotomy compared to central or proximal

pancreatotomy (56,57) while pancreas head resection did not alter glucose homeostasis at 12 months (57,58). Evidence in patients with T1D remains more arguable. Nonetheless, recent reports in patients with T1D suggested an increased loss of β cells in the dorsal lobe of the pancreas (59) while another demonstrated similar β-cell mass in both pancreas tail and body (48). Topographic analysis of the pancreas using multiparametric MRI may thus be an additional poorly invasive tool to improve the prediction of pancreatic functions evolution during the first year of T1D.

Our study has several strengths. It is the first MRI study that evaluates the topography of pancreas atrophy and the presence of age-related differences in the exocrine pancreas in a cohort including 50% of prepubertal patients. Longitudinal assessment of MRI-related indices and pancreatic functions allowed both cross-sectional comparisons and the development of prediction models of pancreatic functions.

This study was limited by the influence of stomach repletion on pancreatic measures as MRI scans were performed in a non-fasting state to decrease the risk of hypoglycemia during the imaging session. The impact of this bias was limited as a recent study demonstrated no influence of prandial state on pancreatic measurements (21). As there is no clear anatomical consensus on the limits between the pancreas body and tail, the limits described in previous studies were applied strictly (36,37). The size of the cohort was small. Therefore, further validation on a larger sample size is advised. This study however regroups one of the biggest cohorts of pediatric

new-onset T1D patients assessing both pancreas imaging and pancreatic functions (9).

In conclusion, children with new-onset T1D demonstrated an age-related homogenous reduction of PV with prepubertal patients having increased atrophy compared to controls. Topographic correlations between pancreatic subregion indices and their respective residual functions, both early after the diagnosis and after the first year of diabetes, were observed. Moreover, these pancreatic MRI-derived parameters improved prediction models of endocrine function during the first year of type 1 diabetes, especially when analyzed topographically. Finally, different PV evolution patterns after clinical T1D onset were observed that were correlated with pancreas atrophy at diagnosis but not with common markers of glucose homeostasis. We believe that *in-situ* analysis of T1D pancreas using MRI might improve the understanding of the heterogeneity observed within patients and provide additional non-invasive imaging markers of pancreatic functions.

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3.6.6 References

1. Mallone R, Eizirik DL. Presumption of innocence for beta cells: why are they vulnerable autoimmune targets in type 1 diabetes? Diabetologia. 2020 Oct 7;63(10):1999–2006.
2. Battaglia M, Ahmed S, Anderson MS, Atkinson MA, Becker D, Bingley PJ, et al. Introducing the endotype concept to address the challenge of disease heterogeneity in type 1 diabetes. Diabetes Care. 2020;43(1):5–12.
3. Jacobsen LM, Bocchino L, Evans-Molina C, DiMeglio L, Goland R, Wilson DM, et al. The risk of progression to type 1 diabetes is highly variable in individuals with multiple autoantibodies following screening. Diabetologia. 2020 Mar 1;63(3):588–96.
4. Fonolleda M, Murillo M, Vázquez F, Bel J, Vives-Pi M. Remission Phase in Paediatric Type 1 Diabetes: New Understanding and Emerging Biomarkers. Horm Res Paediatr. 2017;88(5):307–15.
5. Sims EK, DiMeglio LA. Cause or effect? A review of clinical data demonstrating beta cell dysfunction prior to the clinical onset of type 1 diabetes. Mol Metab. 2019 Sep 1;27:S129–38.
6. Wei Q, Qi L, Lin H, Liu D, Zhu X, Dai Y, et al. Pathological Mechanisms in Diabetes of the Exocrine Pancreas: What's Known and What's to Know. Vol. 11, Frontiers in Physiology. Frontiers Media S.A.; 2020.
7. Vecchio F, Messina G, Giovenzana A, Petrelli A. New Evidence of Exocrine Pancreatopathy in Pre-symptomatic and Symptomatic Type 1 Diabetes. Vol. 19, Current Diabetes Reports. Current Medicine Group LLC 1; 2019. p. 1–8.
8. Jones CM, Castle WB, Mulholland HB, Bailey F. Pancreatic and hepatic activity in diabetes mellitus: The alterations with some observations on the etiology of the disease. Arch Intern Med. 1925 Mar 1;35(3):315–36.
9. Foster TP, Bruggeman B, Campbell-Thompson M, Atkinson MA, Haller MJ, Schatz DA. Exocrine pancreas dysfunction in type 1

diabetes. Endocr Pract. 2020 Dec 1;26(12):1505–13.

10. Campbell-Thompson ML, Filipp SL, Grajo JR, Nambam B, Beegle R, Middlebrooks EH, et al. Relative pancreas volume is reduced in first-degree relatives of patients with type 1 diabetes. Diabetes Care. 2019;42(2):281–7.

11. Panicot L, Mas E, Thivolet C, Lombardo D. Circulating antibodies against an exocrine pancreatic enzyme in type 1 diabetes. Diabetes. 1999 Dec 1;48(12):2316–23.

12. Hardt PD, Ewald N, Bröckling K, Tanaka S, Endo T, Kloer HU, et al. Distinct autoantibodies against exocrine pancreatic antigens in European patients with type 1 diabetes mellitus and non-alcoholic chronic pancreatitis. Vol. 9, Journal of the Pancreas. 2008. p. 683–9.

13. de Boer P, Pirozzi NM, Wolters AHG, Kuipers J, Kusmartseva I, Atkinson MA, et al. Large-scale electron microscopy database for human type 1 diabetes. Nat Commun. 2020 Dec 1;11(1):1–9.

14. Di Cesare E, Previti M, Lombardo F, Mazzù N, Di Benedetto A, Cucinotta D. Prevalence of autoantibodies to carbonic anhydrase II and lactoferrin in patients with type 1 diabetes. In: Annals of the New York Academy of Sciences. John Wiley & Sons, Ltd; 2004. p. 131–2.

15. Virostko J, Williams J, Hilmes M, Bowman C, Wright JJ, Du L, et al. Pancreas volume declines during the first year after diagnosis of type 1 diabetes and exhibits altered diffusion at disease onset. Diabetes Care. 2019;42(2):248–57.

16. Sims EK, Syed F, Nyalwidhe J, Bahnson HT, Haataja L, Speake C, et al. Abnormalities in proinsulin processing in islets from individuals with longstanding T1D. Transl Res. 2019;213:90–9.

17. Leete P, Oram RA, McDonald TJ, Shields BM, Ziller C, Roep BO, et al. Studies of insulin and proinsulin in pancreas and serum support the existence of aetiopathological endotypes of type 1 diabetes associated with age at diagnosis. Diabetologia. 2020;63(6):1258–67.

18. Parviainen A, Härkönen T, Ilonen J, But A, Knip M. Heterogeneity of

Type 1 Diabetes at Diagnosis Supports Existence of Age-Related Endotypes. Diabetes Care. 2022 Apr 1;45(4):871–9.

19. Dufort MJ, Greenbaum CJ, Speake C, Linsley PS. Cell type-specific immune phenotypes predict loss of insulin secretion in new-onset type 1 diabetes. JCI insight. 2019 Feb 21;4(4).

20. Bogun MM, Bundy BN, Goland RS, Greenbaum CJ. C-peptide levels in subjects followed longitudinally before and after type 1 diabetes diagnosis in trialnet. Diabetes Care. 2020 Aug 1;43(8):1836–42.

21. Williams JM, Hilmes MA, Archer B, Dulaney A, Du L, Kang H, et al. Repeatability and Reproducibility of Pancreas Volume Measurements Using MRI. Sci Rep. 2020;10(1):1–7.

22. Gaglia JL, Harisinghani M, Aganj I, Wojtkiewicz GR, Hedgire S, Benoist C, et al. Noninvasive mapping of pancreatic inflammation in recent-onset type-1 diabetes patients. Proc Natl Acad Sci. 2015;112(7):2139–44.

23. Garcia TS, Rech TH, Leitão CB. Pancreatic size and fat content in diabetes: A systematic review and meta-analysis of imaging studies. PLoS One. 2017;12(7):1–15.

24. Goda K, Sasaki E, Nagata K, Fukai M, Ohsawa N, Hahafusa T. Pancreatic volume in type 1 and type 2 diabetes mellitus. Acta Diabetol. 2001;38(3):145–9.

25. Regnell SE, Peterson P, Trinh L, Broberg P, Leander P, Lernmark, et al. Pancreas volume and fat fraction in children with Type 1 diabetes. Diabet Med. 2016;33(10):1374–9.

26. Campbell-Thompson M, Wasserfall C, Montgomery EL, Atkinson MA, Kaddis JS. Pancreas organ weight in individuals with disease-associated autoantibodies at risk for type 1 diabetes. Vol. 308, JAMA. JAMA; 2012. p. 2337–9.

27. Augustine P, Gent R, Louise J, Taranto M, Penno M, Linke R, et al. Pancreas size and exocrine function is decreased in young children with recent-onset Type 1 diabetes. Diabet Med. 2020 Aug 17;37(8):1340–3.

28. Ross JJ, Wasserfall CH, Bacher R, Perry DJ, McGrail K, Posgai AL, et al. Exocrine Pancreatic Enzymes Are a Serological Biomarker for Type 1 Diabetes Staging and Pancreas Size. Diabetes. 2021 Apr 1;70(4):944–54.

29. Pollé OG, Delfosse A, Martin M, Louis J, Gies I, den Brinker M, et al. Glycemic Variability Patterns Strongly Correlate With Partial Remission Status in Children With Newly Diagnosed Type 1 Diabetes. Diabetes Care. 2022;45(October):2360–8.

30. Besser REJ, Bell KJ, Couper JJ, Ziegler A-G, Wherrett DK, Knip M, et al. <scp>ISPAD</scp> Clinical Practice Consensus Guidelines 2022: Stages of type 1 diabetes in children and adolescents. Pediatr Diabetes. 2022 Dec 30;23(8):1175–87.

31. Harris PA, Taylor R, Minor BL, Elliott V, Fernandez M, O'Neal L, et al. The REDCap consortium: Building an international community of software platform partners. J Biomed Inform. 2019;95(May):103208.

32. Wentworth JM, Bediaga NG, Giles LC, Ehlers M, Gitelman SE, Geyer S, et al. Beta cell function in type 1 diabetes determined from clinical and fasting biochemical variables. Diabetologia. 2019;62(1):33–40.

33. Wang J, Bediaga N, Mallone R, Larger E, Harrison LC, Wentworth JM, et al. Validation in the general population of a C-peptide estimate equation to measure beta cell function in recent-onset type 1 diabetes. Acta Diabetologica. 2020.

34. Mortensen HB, Hougaard P, Swift P, Hansen L, Holl RW, Hoey H, et al. New definition for the partial remission period in children and adolescents with type 1 diabetes. Diabetes Care. 2009;32(8):1384–90.

35. Steinkohl E, Olesen SS, Mark EB, Hansen TM, Frandsen LK, Drewes AM, et al. Progression of parenchymal and ductal findings in patients with chronic pancreatitis: A 4-year follow-up MRI study. Eur J Radiol. 2020;125.

36. Fukushima H, Itoh S, Takada A, Mori Y, Suzuki K, Sawaki A, et al. Diagnostic value of curved multiplanar reformatted images in multislice CT for the detection of resectable pancreatic ductal

adenocarcinoma. Eur Radiol. 2006;16(8):1709–18.

37. Barral M, Soyer P, Ben Hassen W, Gayat E, Aout M, Chiaradia M, et al. Diffusion-weighted MR imaging of the normal pancreas: Reproducibility and variations of apparent diffusion coefficient measurement at 1.5-and 3.0-Tesla. Diagn Interv Imaging. 2013;94(4):418–27.

38. Virostko J, Hilmes M, Eitel K, Moore DJ, Powers AC. Use of the electronic medical record to assess pancreas size in type 1 diabetes. PLoS One. 2016 Jul 1;11(7):158825.

39. Statistical Methods in Medical Research. Stat Methods Med Res. 2002 Jan 1;

40. Wang X, Misawa R, Zielinski MC, Cowen P, Jo J, Periwal V, et al. Regional Differences in Islet Distribution in the Human Pancreas - Preferential Beta-Cell Loss in the Head Region in Patients with Type 2 Diabetes. PLoS One. 2013;8(6):e67454.

41. Diedisheim M, Mallone R, Boitard C, Larger E. β-cell mass in nondiabetic autoantibody-positive subjects: An analysis based on the network for pancreatic organ donors database. J Clin Endocrinol Metab. 2016;101(4):1390–7.

42. Rodriguez-Calvo T, Ekwall O, Amirian N, Zapardiel-Gonzalo J, Von Herrath MG. Increased immune cell infiltration of the exocrine pancreas: A possible contribution to the pathogenesis of type 1 diabetes. Diabetes. 2014 Nov 1;63(11):3880–90.

43. Nigi L, Maccora C, Dotta F, Sebastiani G. From immunohistological to anatomical alterations of human pancreas in type 1 diabetes: New concepts on the stage. Vol. 36, Diabetes/Metabolism Research and Reviews. John Wiley and Sons Ltd; 2020. p. e3264.

44. Alexandre-Heymann L, Mallone R, Boitard C, Scharfmann R, Larger E. Structure and function of the exocrine pancreas in patients with type 1 diabetes. Vol. 20, Reviews in Endocrine and Metabolic Disorders. Springer New York LLC; 2019. p. 129–49.

45. Vecchio F, Messina G, Giovenzana A, Petrelli A. New Evidence of

Exocrine Pancreatopathy in Pre-symptomatic and Symptomatic Type 1 Diabetes. Vol. 19, Current Diabetes Reports. Current Medicine Group LLC 1; 2019.

46. Wright JJ, Saunders DC, Dai C, Poffenberger G, Cairns B, Serreze D V., et al. Decreased pancreatic acinar cell number in type 1 diabetes. Diabetologia. 2020 Jul 9;63(7):1418–23.

47. Atkinson MA, Campbell-Thompson M, Kusmartseva I, Kaestner KH. Organisation of the human pancreas in health and in diabetes. Diabetologia. 2020;63(10):1966–73.

48. Damond N, Engler S, Zanotelli VRT, Schapiro D, Wasserfall CH, Kusmartseva I, et al. A Map of Human Type 1 Diabetes Progression by Imaging Mass Cytometry. Cell Metab. 2019;29(3):755-768.e5.

49. Vecchio F, Buono N Lo, Stabilini A, Nigi L, Dufort MJ, Geyer S, et al. Abnormal neutrophil signature in the blood and pancreas of presymptomatic and symptomatic type 1 diabetes Federica Vecchio , … , the Type 1 Diabetes TrialNet Study Group , Manuela Battaglia Find the latest version : Abnormal neutrophil signature in the bl. JCI Insight. 2018;3(18):1–17.

50. Foster TP, Bruggeman B, Campbell-Thompson M, Atkinson MA, Haller MJ, Schatz DA. Exocrine pancreas dysfunction in type 1 diabetes. Vol. 26, Endocrine Practice. 2020. p. 1505–13.

51. Campbell-Thompson ML, Filipp SL, Grajo JR, Nambam B, Beegle R, Middlebrooks EH, et al. Relative pancreas volume is reduced in first-degree relatives of patients with type 1 diabetes. Diabetes Care. 2019 Feb 1;42(2):281–7.

52. Sims EK, Chaudhry Z, Watkins R, Syed F, Blum J, Ouyang F, et al. Elevations in the fasting serum Proinsulin-to-C-peptide ratio precede the onset of type 1 diabetes. Diabetes Care. 2016;39(9):1519–26.

53. Inshaw JRJ, Cutler AJ, Crouch DJM, Wicker LS, Todd JA. Genetic variants predisposing most strongly to type 1 diabetes diagnosed under age 7 years lie near candidate genes that function in the immune system and in pancreatic B-cells. Diabetes Care. 2020 Jan 1;43(1):169–77.

54. Philippe MF, Benabadji S, Barbot-Trystram L, Vadrot D, Boitard C, Larger E. Pancreatic volume and endocrine and exocrine functions in patients with diabetes. Pancreas. 2011;40(3):359–63.

55. Sasamori H, Fukui T, Hayashi T, Yamamoto T, Ohara M, Yamamoto S, et al. Analysis of pancreatic volume in acute-onset, slowly-progressive and fulminant type 1 diabetes in a Japanese population. J Diabetes Investig. 2018;9(5):1091–9.

56. Wu L, Nahm CB, Jamieson NB, Samra J, Clifton-Bligh R, Mittal A, et al. Risk factors for development of diabetes mellitus (Type 3c) after partial pancreatectomy: A systematic review. Clin Endocrinol (Oxf). 2020;92(5):396–406.

57. Menge BA, Schrader H, Breuer TGK, Dabrowski Y, Uhl W, Schmidt WE, et al. Metabolic consequences of a 50% partial pancreatectomy in humans. Diabetologia. 2009;52(2):306–17.

58. Hempel S, Oehme F, Ehehalt F, Solimena M, Kolbinger FR, Bogner A, et al. The Impact of Pancreatic Head Resection on Blood Glucose Homeostasis in Patients with Chronic Pancreatitis. J Clin Med. 2022;11(3):663.

59. Campbell-Thompson ML, Kaddis JS, Wasserfall C, Haller MJ, Pugliese A, Schatz DA, et al. The influence of type 1 diabetes on pancreatic weight. Diabetologia. 2016;59(1):217–21.

3.6.7 Supplementary material

Supplementary material

Supplementary Table 1. Summary of technical parameters of MRI sequences.

Sequence	Field-of-view (Frequency x Phase, cm)	Matrix (Frequency x Phase)	Slice Thickness/gap (mm)	TE/TR (ms)	#slices	Breath-holds	Total duration
T2-SSFSE**	30 x 30	400 x 224	3/0	85/525	40	2	0 min 22 s
T1-SPGR**	32 x 29	292 x 256	3.4/0	1.1/4.1	84	1	0 min 13 s
DWI¶	36 x 36	128 x 160	4/1	50/7058*	40	0	3 min 04 s*
Multi-echo GRE	44 x 44	160 x 160	5/0	0.9-4.3/5.8	56	1	0 min 16 s

*These values of TR and acquisition duration are hypothetical given that they were dependent on the respiratory rate. The given values are for a respiratory rate of 17/min. The body RF-coil was used for transmission and multi-array coils (in the table and flexible AIR coils over the patient) for reception. ** Flip angle 12°.¶ Two averages were used for diffusion encoded images b=50 s/mm² and six for b=800 s/mm² and parallel imaging (ASSET/SENSE) with an acceleration factor of 2. To minimize breathing-induced motion, respiratory gating was employed with a navigator placed at the top of the liver dome.
TE: time of echo; TR: time of repetition.

Supplementary Table 2. Pancreas volume (PV) and pancreas index (PI) measurements *per* disease and *per* puberty status.

Pancreas volume indices		Control		
		Puber	**Prepuber**	**difference**
PV (mL)	Head	20.7 [18.5; 22.9]	15.1 [11.6; 18.5]	+5.62 [+1.75; +9.51]**‡
	Body+Tail	29.6 [25.3; 34.0]	20.3 [17.0; 23.5]	+9.39 [+4.15; +14.6]***‡
	Whole pancreas	50.4 [45.1; 55.6]	35.4 [29.0; 41.8]	+15.0 [+7.12; +22.8]***‡
PI (mL/kg)	Head	0.43 [0.36; 0.50]	0.54 [0.43; 0.65]	-
	Body+Tail	0.60 [0.52; 0.67]	0.73 [0.63; 0.83]	-0.13 [-0.25; -0.02]*‡
	Whole pancreas	0.99 [0.89; 1.07]	1.27 [1.07; 1.47]	-
Pancreas volume indices		**T1D**		
		Puber	**Prepuber**	**difference**
PV (mL)	Head	13.3 [10.0; 16.5]	8.13 [7.29; 9.70]	+5.45 [+0.69; .7.84]*†
	Body+Tail	18.01 [14.2; 21.8]	9.36 [7.38; 14.9]	+8.12 [+0.98; +11.7]*†
	Whole pancreas	27.8 [22.6; 33.0]	15.5 [13.6; 24.8]	+10.2 [+3.50; +15.8]*†
PI (mL/kg)	Head	0.26 [0.20; 0.32]	0.31 [0.26; 0.37]	-
	Body+Tail	0.35 [0.29; 0.42]	0.39 [0.30; 0.47]	-
	Whole pancreas	0.55 [0.46; 0.64]	0.63 [0.52; 0.74]	-
		difference	**difference**	
		+7.45 [+3.62; +11.3]***‡	+5.33 [+1.10; +11.1]**†	
		+11.6 [+6.12; +17.1]***‡	+8.47 [+3.36; +13.4]**†	
		+22.6 [+15.5; +29.7]***‡	+15.2 [+7.85; +26.3]***†	
		+0.17 [+0.08; +0.25]***‡	+0.23 [+0.10; +0.35]**‡	
		+0.25 [+0.15; +0.34]***‡	+0.34 [+0.22; +0.46]***‡	
		+0.44 [+0.30; +0.62]***†	+0.64 [+0.43; +0.86]***‡	

Differences are calculated as follows: Puber - Prepuber, and Control - DT1 (expressed in ml for PV and in % for PI)
‡Mean difference from t-test, †Median difference from Mann-Withney U test. Abbreviations : PI= pancreas index, PV = pancreas volume, T1D = type 1 diabetes. The level of significance of the differences is represented as follows: p<0.05 (*), p<0.01 (**), p<0.001 (***).

Supplementary Table 3. Correlations between PV indices and pancreatic functions

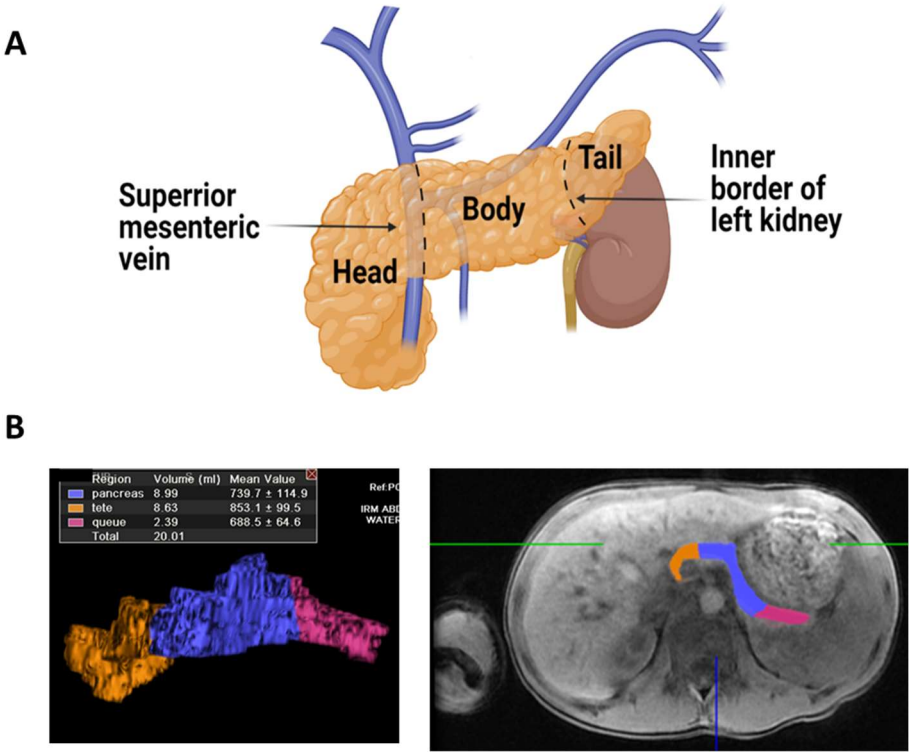
	Pancreas volume (mL)				Pancreas Index (mL/kg)			
	Whole	***Head***	***Body***	***Tail***	***Whole***	***Head***	***Body***	***Tail***
Endocrine (n=31)								
HbA_{1C} — %	/	/	/	/	/	/	/	/
Insulin doses (IU/kg/day)	/	/	/	/	/	/	/	/
$IDAA_{1C}$	/	/	/	/	/	/	/	/
$CPEP_{EST}$ §	/	/	/	R=0.55, p<0.001	/	/	/	/
$CPEP_{BASAL}$ §	/	/	/	R=0.5, p<0.01	/	/	/	/
Exocrine								
Trypsinogen (ng/mL) -- n=31	R=0.46, p=0.009	R=0.49, p=0.006	/	/	R=0.52, p=0.002	R=0.49, p=0.005	/	/
Lipase (UI/L) -- n=26	R=0.45, p=0.022	R=0.38, p=0.056	/	/	R=0.58, p=0.002	R=0.45, p=0.021	/	/

*Correlations were calculated using Spearman method. § measured at 3 months postdiagnosis. * Calculated according to Wentworth et al* (267) *Abbreviations :* R: Spearman *rho*

Supplementary Table 4. Summary of most interesting bivariate predictive models of glucose homeostasis during the first year of T1D.

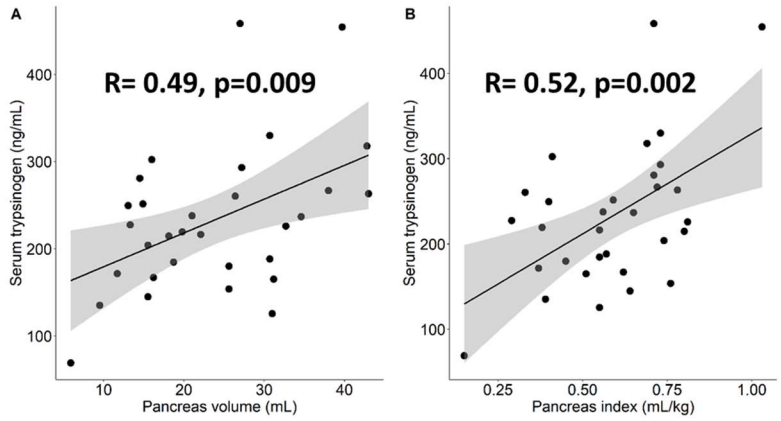
Glucose homeostasis parameter	*Time-point (months)*	*Parameter 1*	*Parameter 2*	*Regression coefficient (r^2)*	*p value*	*Clinical meaning*
$CPEP_{EST}$	3	**mean ADC_{HEAD}**	**Puberty**	0.42	0.0008	Postpubertal and increased ADC predict higher $CPEP_{EST}$ at 3 months
$CPEP_{EST}$	3	**PV_{TAIL}**	**Sex**	0.38	0.002	Bigger PV_{TAIL} and male gender predict higher $CPEP_{EST}$ at 3 months
$CPEP_{EST}$	12	**PV_{TAIL}**	**Sex**	0.42	0.003	Bigger PV_{TAIL} and male gender predict higher $CPEP_{EST}$ at 12 months
$CPEP_{BASAL}$	3	**PV_{TAIL}**	**Sex**	0.42	0.0007	Bigger PV_{TAIL} and male gender predict higher CPEPBASAL at 3 months
$IDAA_{1C}$	3	**mean ADC_{BODY}**	**Trypsinogen**	0.41	0.0008	Increase of ADC and Trypsinogen predict higher $IDAA_{1C}$ at 3 months
$IDAA_{1C}$	6	**mean ADC_{BODY}**	**STD ADC_{BODY}**	0.46	0.0004	Increase of ADC_{mean} with low ADC SD predict higher IDAA1C at 6 months
TDD	3	**mean ADC_{BODY}**	**Trypsinogen**	0.38	0.0014	Increase of ADC_{mean} and trypsinogen predict higher TDD at 3 months
TDD	6	**mean ADC_{BODY}**	**Trypsinogen**	0.45	0.0006	Increase of ADC_{mean} and trypsinogen predict higher TDD at 6 months
TDD	9	**mean ADC_{BODY}**	**Trypsinogen**	0.34	0.005	Increase of ADC_{mean} and trypsinogen predict higher TDD at 9 months
HbA_{1C}	3	**mean FF_{HEAD}**	**Puberty**	0.5	0.0001	Prepubertal and lower pancreatic FF predicted higher HbA_{1C} at 3 months
HbA_{1C}	3	**mean FF_{WHOLE}**	**Puberty**	0.43	0.0005	Prepubertal and lower pancreatic FF predicted higher HbA_{1C} at 3 months

Supplementary Figure 1



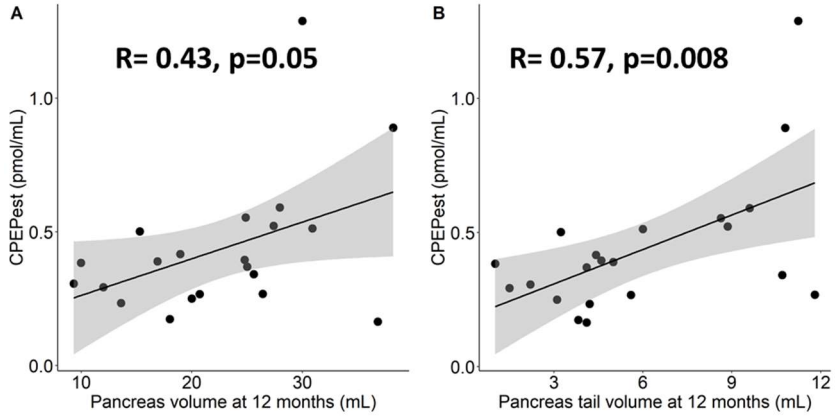
Supplementary Fig. 1: Visualization of pancreas subregions delimitations. (**A**) Graphical/Schematic representation of pancreas division into three subregions (i.e., head, body and tail) according to predefined anatomical limits (dashed lines). (**B**) Pancreas subregions volumetry (left panel) of a nine-years-old girl with new-onset type 1 diabetes measured using Vitrea^{TM} software on anatomic MRI sequence (right panel). MRI: magnetic resonance imaging.

Supplementary Figure 2



Supplementary Figure 2: Linear regressions between PV (**A**) and PI (**B**), and serum trypsinogen at clinical onset of T1D. Shaded zone around regression lines represent 95% confidence interval. PI: pancreas index; PV: pancreas volume; R: Spearman *rho*; T1D: type 1 diabetes.

Supplementary Figure 3



Supplementary Figure 3: Linear regressions between PV (**A**) and PV_{TAIL} (**B**), and $CPEP_{EST}$ at 12 months postdiagnosis. Shaded zone around regression lines represent 95% confidence interval. PI: pancreas index; PV: pancreas volume; R: Spearman *rho*; T1D: type 1 diabetes. $CPEP_{EST}$ was calculated according to Wentworth *et al* (267).

3.7 Paper IV: Plasma proteomics in children with new-onset type 1 diabetes: a tool with strong leverage for identification of partial remission biomarkers

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Short running title: plasma proteomics in new-onset type 1 diabetes children.

3.7.1 Introduction

Type 1 diabetes (T1D) is the most prevalent chronic disease in children characterized by an immune-mediated attack against the β cells with various extents of pancreas inflammation (1). In the weeks to years following the initiation of insulitis, patients experience a progressive and asymptomatic demise of insulin-secreting cells (2). Multiple individual cofactors (e.g., immunity, genetics, inflammation, metabolic stress, and β-cell sensitivity to stress) influence the degree and kinetics of β-cell decline (2). When β-cell function falls by eighty percent, patients experience a clinical state of insulinopenia with subsequent global metabolic disbalance (e.g., dyslipidemia, hyperglycemia, and eventually acidosis) and appearance of insulinopenia-related symptoms (i.e., polyuria, polydipsia, and weight loss), corresponding to clinical onset of the disease (1,3,4).

Therapeutic advances of the past twenty years (e.g., glucose monitoring systems, insulin analogs, and delivery systems) brought patients with T1D supporting tools that enhanced day-to-day management and helped improve glycemic control (1,5). To date, however, less than 30% of patients with T1D achieve recommended glycemic targets (6,7). Also, an incompressible prevalence of acute and chronic complications remains in these patients (5,8–11) with consequent decrease in quality of life and reduction in lifespan expectancy (12,13). Currently, disease-modifying therapies are missing as gaps remain in the understanding of T1D heterogeneity, holding patients to lifelong exogenous insulin substitution therapy (14,15). Current efforts, such as the DIATAG consortium (16), focus

on a better characterization of T1D heterogeneity to achieve precision medicine (14–19).

One specific example of T1D variable clinical evolution is the occurrence of partial remission (PR), in approximately 50% of new-onset patients, shortly after the initiation of insulinotherapy. PR corresponds to a transient recovery of β-cell function that is clinically characterized by increased glycemic stability and low daily exogenous insulin requirements (20). Mechanisms underlying PR remain controversial with recent studies suggesting a combination of improved insulin sensitivity, a relative recovery of immunotolerance against β cells, and a partial recovery of β-cell function (21–23). Owing to the attempt at preserving residual β-cell function after T1D onset, favorable metabolic and immune conditions surrounding PR support the intervention-prone character of the period. Additionally, studies recently highlighted the positive duration-dependent residual effect of PR on short- (i.e., DIABHONEY study) and long-term glucose homeostasis (24). While being a theoretically optimal period, prevention therapies aiming to extend PR yielded short-lasting effects and patchy responses among patients with new-onset T1D. This mostly results from the lack of reliable predictive (bio)markers to perform accurate stratification of patients at diagnosis (14,25).

In-depth profiling of blood-derived specimens (i.e., plasma or serum) using mass-spectrometry approach allows the simultaneous quantification of hundreds of circulating proteins (26,27). While similar strategies were previously applied for biomarker discovery in autoimmune or inflammatory

diseases, a recent review highlighted that only a very small amount of these studies included pediatric patients with only one study performed in plasma of patients with T1D (28,29)[,]. Furthermore, proteomic studies in the T1D field mostly focused on the earliest stages of the disease (i.e., presymptomatic) (28) or on distinguishing patients with T1D from healthy controls (26). Focusing on clinical diversity (e.g., PR occurrence) rather than disease status (i.e., T1D vs healthy controls) may provide new insights into the comprehension of T1D heterogeneity and the identification of new stratification biomarkers.

In the present study, we performed a deep proteomic characterization of plasma of children with new-onset T1D to identify biomarkers present at diagnosis and with a potential to predict the occurrence of PR 3 months postdiagnosis. Various data enrichment analyses were run on identified candidates to unravel pathways underlying PR.

3.7.2 Material and Methods

Study population and design

This is a subsidiary study of the multicentric DIAbetes TAGging (DIATAG) study that was previously described (16). Briefly, patients aged between 6 months to 18 years old were recruited at clinical diagnosis of T1D that was defined by the International Society for Pediatric and Adolescent Diabetes (ISPAD) criteria (30). Patients were positive for at least one anti-islet autoantibody (i.e., anti-insulin, anti-insulinoma-associated antigen-2, anti-glutamic acid decarboxylase, anti-Zinc transporter 8). Informed written consent was obtained for every parent, and patient when aged above 7 years old. The study protocol was approved by the seven participating ethical committees (Comité d'Ethique Hospitalo-Facultaire of CUSL (2018/04DEC/462)) and is registered on www.clinicaltrial.gov (NCT04007809). Exclusion criteria are described elsewhere (NCT04007809).

Demographic data (i.e., age at diagnosis, sex, weight, height, and BMI) and diabetes characteristics (i.e., presence of diabetic ketoacidosis at diagnosis, anti-islet antibodies, insulin regimen) were collected at diagnosis. Tanner's stage was determined during the initial hospitalization for diabetes by a pediatric endocrinologist that classified the participants as prepubertal (Tanner I) or pubertal (Tanner II to V) (31). When data obtained during the clinical examination were borderline (e.g., early M2 or testicular volume 4-6mL) or absent, serum level of LH was measured on the baseline blood test where LH>0.3 UI/L defined puberty onset. The baseline screening (i.e., blood draw, urine, and saliva) was performed after an overnight fast

between 5 to 21 days after diagnosis, to allow metabolic stabilization. From diagnosis, clinical parameters (i.e., Insulin-Dose Adjusted. A1C [$IDAA_{1C}$], HbA_{1C}, total insulin daily dose [TDD]) were collected at each outpatient clinical visit (i.e., every 3 months) for 1 year. All data were gathered inside the Research Electronic Data Capture (REDCap) system provided by Vanderbilt University (Nashville, USA) and hosted at Cliniques universitaires Saint-Luc (CUSL).

Baseline plasma samples from sixteen patients with T1D from the DIATAG cohort were used for this study. These were carefully selected according to the patient's remission status at +3 months, pubertal status, gender, and participating center to reduce the influence of cofactors on plasma proteome analysis (i.e., age, gender, preanalytic management of the samples).

Determination of the remission status

Remission status was defined using the insulin dose-adjusted $A1_C$ ($IDAA_{1C}$) score, as follows: HbA1c (%) + (4 × insulin dose (U/kg body weight per 24 h) (32), with a score above 9 defining nonremitters and a score below 9 defining remitters. TDD was either reported by patients (i.e., MDI users) or calculated using the software for pump users.

Blood samples collection

Venous whole blood was collected in EDTA tubes for plasma (Monovette®, Sarstedt, Nümbrecht, Germany). Within the 90 minutes of the sampling, collection tubes were centrifugated 15 minutes at 2000G

at 4°C to achieve poor platelet plasma. The upper layer corresponding to plasma was slowly collected to avoid contamination by red blood cells (i.e., leaving the first 5mm in the tube), and aliquoted and frozen at -80°C in the biobank until further use. The sample collection and preanalitycal management of the samples were standardized in all participating centers.

Plasma samples preparation for proteomics

All samples were prepared in parallel and with the same batch of reagents. Plasma samples were thawed on ice before being centrifuged for 15 minutes at 14.000G to remove impurities. Plasma (1 mL) from each patient was depleted using ProteoMiner Protein Enrichment Kit (Bio-Rad) according to the manufacturer's instructions (33,34). We performed a volume-based normalizing method on the input as (1) human plasmatic proteins remain in a tight concentration range (35) and (2) translation of the method to the clinic will be easier. Proteominer beads were washed three times before being eluted defining the eluate fraction. The eluate was collected and proteins were quantified using the Bradford method (36). The depleted proteins (i.e., 250 μg) were further reduced and alkylated in low-bind Eppendorf tubes (Eppendorf LoBind) as previously described (37), before undergoing cold acetone precipitation overnight (i.e., -20°C, 4:1 volume). Proteins were washed twice using cold acetone, resuspended in 50 mM TEAB and digested overnight using trypsin (Promega; Madison, WI) at an enzyme:substrate ratio of 1:100 (37°C under 750 rpm continuous agitation).

The resulting peptides were quantified using Pierce™ kit (Thermo Scientific, Vienna, Austria) according to the manufacturer's instructions.

Peptides from each patient (100µg per patient) were labelled with tandem mass tags (TMT, TMTpro^{TM}16plex, Thermo Scientific, Vienna, Austria) according to the manufacturer's instructions. Samples from all 16 patients were pooled in one final sample that was washed on a C18 column (HyperSep^{TM} 200mg, Thermo Scientific, Vienna, Austria) to remove the excess TMT labeling and improve further analysis (i.e. reducing the background noise).

To further decrease the complexity of the proteome, we fractionated off-line by HPLC the pooled labeled sample into 51 different fractions using hydrophilic interaction liquid chromatography (HILIC) (38,39). These were pooled two by two (i.e., 1+26, 2+27, …25+51) to reduce the number of samples while maintaining distinct hydrophilic characteristics, resulting in a total of 25 fractions (**Figure 1**).

Relative quantification by Liquid Chromatography-Tandem-Mass-Spectrometry (LC-MS/MS)

One µg of peptides dissolved in solvent A (0.1% TFA in 2% ACN) was directly loaded onto a reversed-phase pre-column (Acclaim PepMap 100, Thermo Scientific) and eluted in backflush mode. Peptide separation was achieved using a reversed-phase analytical column (Acclaim PepMap RSLC, 0.075 × 250 mm, Thermo Scientific) with a 140 min linear gradient of 4%–32% solvent B (0.1% TFA in 80% ACN) for 99 min, 32%–60% solvent B for 10 min, 60%–95% for 1 min and holding at 95% for the last 10 min at a constant flow rate of 300 nL/min on an Ultimate 3000 RSLC nanoHPLC system (Thermo Scientific, Vienna, Austria). The peptides were analyzed by an Orbitrap

Fusion Lumos tribrid mass spectrometer (Thermo Fisher Scientific, Vienna, Austria) with enabled advanced peak determination and relative quantification was allowed by either MS^2 or SPS MS^3 scans. Intact peptides were detected in the Orbitrap at a resolution of 120,000 with a scan range m/z from 375 to 1500 and an AGC target of $4x10^5$, maximum injection time was set to 50 ms. A data-dependent procedure of MS/MS scans was applied for the top precursor ions above a threshold ion count of 5×10^3 in the MS survey scan with 60s dynamic exclusion. The total cycle time was set to 3s. For MS^2 identification of peptide sequence and quantification of the TMT reporter ions, MS^2 spectra were acquired in the Orbitrap at a resolution of 50,000 after HCD fragmentation at 35% with an AGC target of 1×10^5 ions and a maximum injection time of 120ms. For MS^3 quantification, MS^2 spectra for identification were first acquired in the Ion Trap after CID fragmentation at 30%, for quantification 10 precursors from the MS^2 scan were synchronously selected (SPS MS^3) for HCD fragmentation at 55%, and the MS^3 spectra was acquired in the Orbitrap at a resolution of 50,000 with an AGC of $2x10^5$ and a maximum injection time of 120ms. MS/MS spectra were exported using the following settings: peptide mass range: 350–5000 Da, minimal total ion intensity: 500.

Protein detection and quantification

For protein identification, MS/MS data were processed using Sequest HT search engine within Proteome Discoverer 2.5 against a human protein reference target-decoy database obtained from Uniprot. Trypsin was specified as the cleavage enzyme, allowing up to 2 missed cleavages, 4

modifications per peptide, and up to 3 charges. Relative quantification of proteins was performed according to the signal of the TMT reporter's ions in MS2 or MS3 spectra. The false discovery rate (FDR) was set at less than 1%. Statistical analysis was performed according to remission status.

Statistical analysis according to remission status

All statistical analyses were performed R (R Core Team [2021]. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL [https://www.R-project.org/]) including principal component analysis (PCA), volcano plots, linear mixed models and enrichment analysis. Preprocessed data (see above) were log-transformed, centered, and scaled to achieve a normal distribution. Influences of various cofactors (i.e., gender, puberty status, center, manipulation operator) were manually visualized on PCA plots. Protein abundance differences according to remission status at +3 months were analyzed both as a categorical (i.e., remitters *vs* nonremitters) or a continuous variable (i.e., $IDAA_{1C}$ score) using linear mixed models using statistical R package Limma (40). Models included the remission status or $IDAA_{1C}$ score as fixed effects, and patient gender and pubertal status as a random intercept. Residuals were visualized on Q-Q plots. The level of significance was considered as p value below 0.05 for every analysis.

Data quality check and data mining for the identification of predictive biomarkers

Potential blood cell-derived contaminations were investigated as previously described (41) by comparing their contaminant lists (e.g., platelet,

erythrocytes or coagulation cascade) to our dataset. Distributions of the latter across our samples were visualized to identify potential outliers.

Enrichment analyses were performed independently using over-representation analysis based on Molecular Signatures Database, and Pathway studio network analysis (www.pathwaystudio.com, Elsevier) on significantly differentially expressed proteins. Pathway studio is a database that allows both non-targeted and targeted investigations on interactions between genes, diseases, drugs, and cells based on a collection of more than 25 million Pubmed abstracts, 1700 full-text journals, and results from 200000 clinical trials.

Unbiased approach to identify the best combination of biomarkers using random forest and spectral vector machine approaches

In order to identify the best combination of biomarkers to predict the remission status at +3 months, predictive modelling was combined to a wrapper feature selection technique called Recursive Feature Elimination (RFE). The underlying predictive models are Random Forest and radial Support Vector Machine (SVM) in a classification context. The models and the feature selection procedure were implemented with the caret R package (42).

Verification of biomarker candidates in raw plasma using targeted LC-MS/MS

The raw plasma samples from each patient (n=15) were diluted using a dilution factor of 1:60 to reach an approximative final concentration

between 1 and 2 mg/dL. Plasma was centrifugated for 15 minutes at 20,000G to remove debris before being reduced, alkylated, precipitated and digested as described here above. Peptides were identified and quantified by a targeted Parallel Reaction Monitoring (PRM) (43) method based on proteotypic peptides (identified in SRM Atlas and Peptide Atlas databases) and their calculated m/z ratio. Between 1 and 4 peptides were targeted per protein of interest. Single run without pre-fractionation was performed for each sample. Peptides were analyzed on the same LC-MS/MS setup as described above with specific settings for PRM acquisition. The Orbitrap analyzer was operated in PRM mode using a resolution of 50.000 for MS1 and 30.000 for targeted MS2 scans, an Automatic Gain Control (AGC) target of $4x10^5$ Ions in MS1 and of $2.5x10^5$ in MS2 with maximum injection time set to Auto. Peptides were selected for MS^2 fragmentation with HCD collision energy set at 30%. Spectra were validated and peptide transitions were quantified using Skyline 32.2.0425 (MacCoss Lab, University of Washington).

Data availability

All raw files were uploaded in PRIDE (Proteome Exchange) with the data set identifier PDxxx loginxxx pwdxxxx (**after submission of the paper**).

3.7.3 Results

Characteristics of study participants

The present study was performed on poor-platelet plasma samples from sixteen pediatric patients of the DIATAG consortium. These samples were collected in five recruiting centers with similar sample preanalytical protocols after overnight fast 5 to 21 days after diagnosis of T1D (see **Material and Method** subsection). Patients were prepubertal (62%) with a mean ± SD age of 9.1 ± 4.2 years at diagnosis. Within this cohort, eight patients were remitters (i.e., $IDAA_{1C}$ score <9 at 3 months postdiagnosis) and eight were non-remitters (i.e., $IDAA_{1C}$ score >9 at 3 months postdiagnosis). Detailed characteristics of the cohort are provided in **Table 1**. Schematic representation of the experimental workflow is presented in **Figure 1**.

Table 1: Study participants characteristics

	Global	***Remitters***	***Non-remitters***	***p-value****
Characteristic	***(N= 16)***	***(N=8)***	***(N= 8)***	
Distribution				
Age — years	9.1 ± 4.2	11.7 ± 2.8	6.5 ± 3.8	0.009†
Sex — Male no. (%)	7 (44)	5 (63)	2 (25)	0.3‡
Pubertal — no. (%)	7 (44)	2 (25)	5 (62.5)	0.3‡
BMI (Z-score)	-0.92 ± 1.22	-0.81 ± 1.43	-1.04 ± 1.01	0.65†
Baseline diabetes characteristics				
HbA_{1C} — % [mmol/mol]	12.2 ± 1.6	12.2 ± 2.0	12.1 ± 1.3	0.88†
Presence of ketoacidosis — no. (%)	5 (31)	1 (13)	4 (50)	0.28‡
Glycaemia — mg/dL	404 ± 162	405 ± 199	404 ± 136	0.99†
Insulin administration				
MDI — no. (%)	11 (70)	8 (100)	3 (37.5)	0.2‡

Glycemic control[‖] (n=16)				
HbA_{1C} — % [mmol/mol]	6.3 ± 1.0	5.5 ± 0.4	7.2 ± 0.7	<0.001[†]
Insulin doses — IU/kg/day	0.6 ± 0.4	0.4 ± 0.2	0.8 ± 0.5	0.07[†]
$IDAA_{1C}$	8.8 ± 2.0	7.2 ± 0.6	10.4 ± 1.6	0.006[†]
Fasting and stimulated C-peptide[‖]				
$CPEP_{BASAL}$ (pmol/mL)	0.2 ± 0.2	0.3 ± 0.2	0.2 ± 0.1	0.1[¶]
$CPEP_{STIM}$ (pmol/mL/min)	0.5 ± 0.2	0.7 ± 0.5	0.3 ± 0.2	0.14[¶]
$CPEP_{EST}$ (pmol/mL)[§]	0.5 ± 0.2	0.6 ± 0.2	0.3 ± 0.1	0.01[†]
Miss values — $CPEP_{EST}$/$CPEP_{STIM}$ (n)	2/3	1/2	1/1	/

Legend: Plus–minus values are means ±SD. Percentages may not total to 100 because of rounding. *p-value calculated between remitters and non-remitters group. Results were considered as significant when under 0.05. [†] Student t-test, [‡] Chi-square, [‖] Parameters evaluated at +3 months after diagnosis, [¶] Wilcoxon-test [§] calculated as described by Wentworth et al (44). Abbreviations: HbA1C = Glycated hemoglobin level, $IDAA_{1C}$ = insulin dose-adjusted A1C, MDI = Multiple Daily Injection, NA = not applicable.

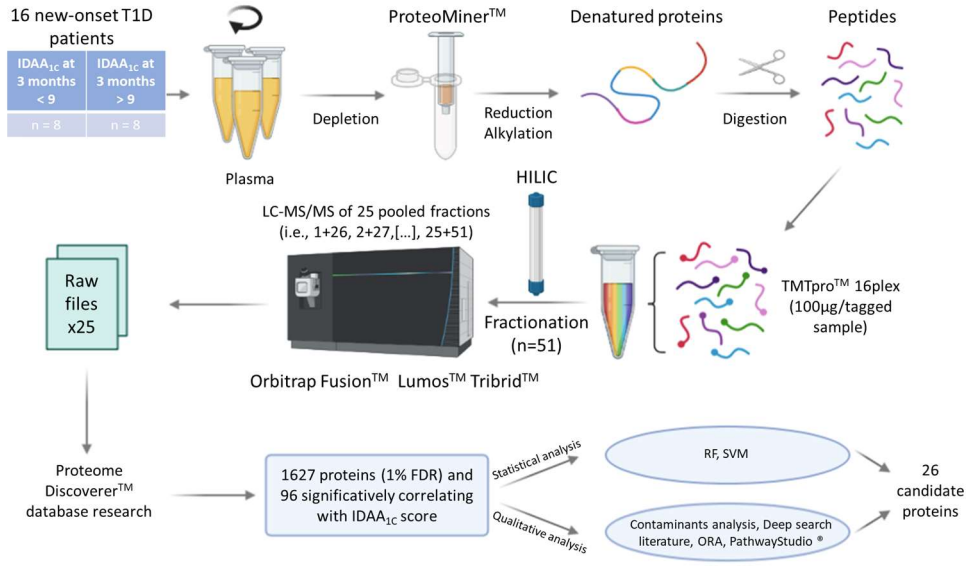

Figure 1: Schematic representation of the experimental workflow. Sixteen plasma samples of patients with new-onset T1D (i.e., between 5-21 days postdiagnosis) were depleted using ProteoMiner™ kit prior to an overnight digestion by trypsin. Resulting peptides were labelled using isobaric TMTpro 16plex™ before being pooled into a single sample and further separeted into 51 fractions using HILIC. Most orthogonal fractions of labelled-peptides were pooled together (i.e., 1+26, 2+27,[…], 25+51) resulting in a total of 25 untargeted LC-MS/MS runs that were subsequently analysed using Proteome Discoverer Software. Correlations between protein relative abundance and $IDAA_{1C}$ score was performed using linear mixed models. Various qualitative and statistical approaches were used to filter best candidates within significant proteins resulting in a total of 26 candidates. Abbreviations : FDR= false discovery rate, HILIC= hydrophilic interaction liquid chromatography, IDAA1C = insulin dose-adjusted A1C, ORA = overrepresentation analysis, RF = random forest, SVM = support vector machine, T1D= type 1 diabetes.

Comprehensive analysis of plasma proteome in patients with T1D yielded the identification and quantification of more than a thousand proteins

To provide an overview of the plasma proteome in patients with new-onset T1D, we performed a comprehensive identification and quantification of depleted plasma from fractionated total peptides. A total of 1627 unique

proteins were identified with an FDR<0.01 using *Proteome Discoverer* ™ software framework. Among them, the great majority were detected and quantified across all plasma samples (i.e., n= 1493/1627, 92%).

Interestingly, crossing our data with HUPO dataset (45), we showed that depletion and fractionation enabled the expansion of the detection range of the mass-spectrometer from $\pm 10^4$ to a magnitude of 10^7 with more than 150 very low plasmatic concentration proteins quantified (i.e., below 10 ng/dL). Furthermore, comparing our data to previous large-scale plasmatic proteomic datasets (46,47), we showed a high overlap between our data and studies from both Liu *et al.* (734/1628) and Keshishian *et al.* (853/1626) (**Fig 2, Venn**). Notably, 660 proteins were solely identified in our experiment.

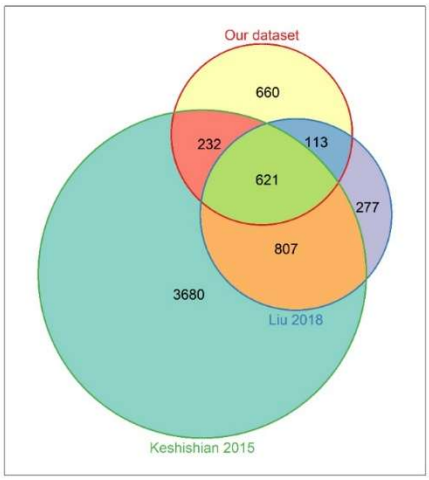

Figure 2: Venn diagram of plasma proteins identified in previous studies (46,47) **and our dataset.** Venn diagram was calculated on reported gene names.

Plasma proteome at diagnosis may improve the prediction of non-remission in newly-onset T1D patients

To investigate whether plasmatic protein levels measured at diagnosis predicted the occurrence of PR at 3 months, data from our untargeted proteomic analysis were analyzed according to PR status either considered as a dichotomic event (i.e., remitter or non-remitter) or a continuous scale (i.e., $IDAA_{1C}$ score). As age and sex influenced plasma proteome in healthy children (47), linear mixed models fitted for gender and pubertal status were used for differential analyses.

When considering PR as a dichotomic event, we found no differentially expressed plasmatic proteins at T1D diagnosis between patients that will undergo PR or not at 3 months (all adjusted p values >0.05). However, as PR demonstrated to be a continuous phenomenon (16), we correlated relative plasmatic concentrations to the $IDAA_{1C}$ score and identified 98 significant candidates (adjusted p values<0.05) (**Fig. 3A**). Most significant proteins distributed asymmetrically in the volcano plot with the great majority showing a positive correlation with $IDAA_{1C}$ score. P-value and protein fold-change (i.e., per unit of $IDAA_{1C}$) of significantly correlated proteins are summarized in **Supplemental Table 1**.

Candidate plasma proteins are not contaminants from the blood cell compartment

As preanalytical treatment of samples may induce bias, dataset was screened for the presence of contaminants as described by Geyer *et al.* (41). Lists of potential contaminant proteins for erythrocytes lysis, coagulation

events, and platelets contamination were retrieved to evaluate the overlap with candidate proteins (41).

While most of proteins listed as platelet, coagulation and erythrolysis contaminant were detected in our dataset (n=25/30, n=30/30 and n=23/30 respectively), these were mostly found in low abundance. Interestingly, none of our proteins of interest were recognized as erythrocyte or coagulation contaminants (**Fig. 3A-B**). Focusing on platelet contamination, 11 of 98 candidates were recognized as potential plasmatic contaminants (i.e., TUBB1, TUBB4B, PARVB, TPM4, CAVIN2, VCL, FERMT3, TLN1, PLEK, TAGLN2, YWHAZ) though rest of platelet contaminants distributed heterogeneously within the volcano plot (i.e., both overexpressed and underexpressed) (**Fig. 3C**).

Deepening the characterization of platelet contamination, we looked at the global distribution of these contaminant proteins in the samples to identify potential outliers and investigate the presence of a global trend in their abundance across $IDAA_{1C}$ values. As shown in **Fig. 3D**, platelet contaminants were homogeneously distributed across all samples (i.e., similar relative abundance) without demonstrating an $IDAA_{1C}$-specific pattern.

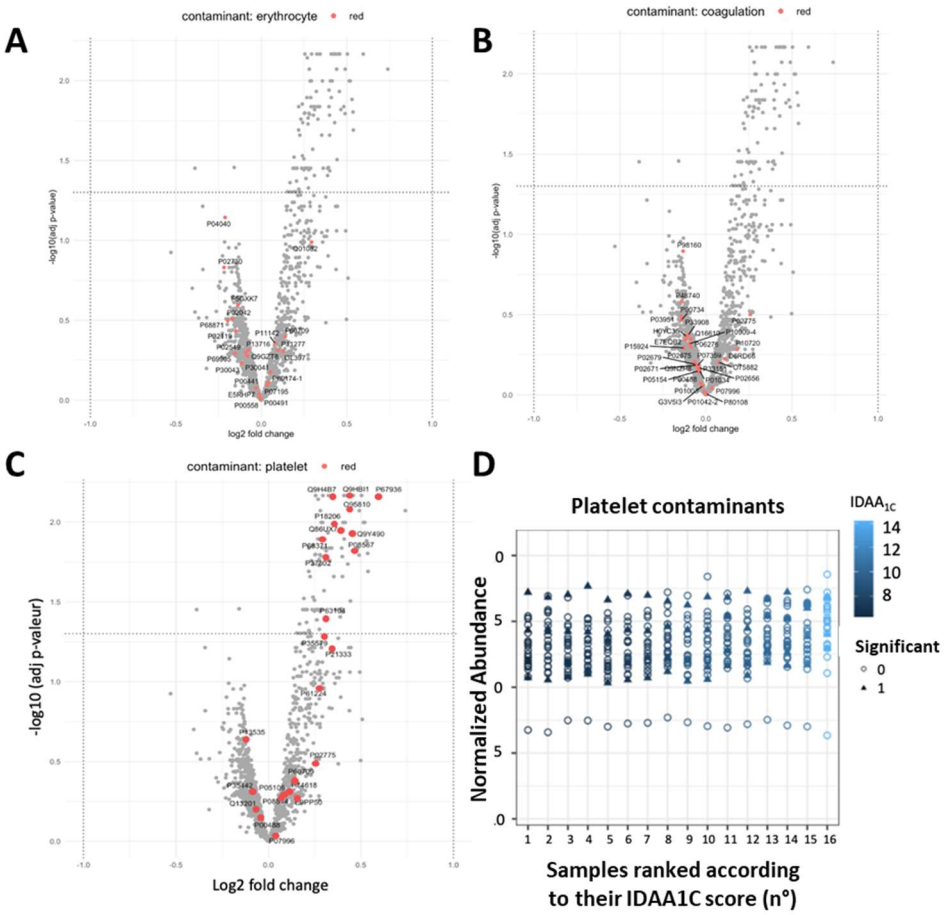

Figure 3: Graphical representation of erythrocyte, coagulation, and platelet plasmatic contamination. Volcano plots of plasma proteins' relative abundance according to $IDAA_{1C}$ score. Red points represent erythrocyte (**A**), coagulation (**B**), and platelet (**C**) contaminants. Dashed horizontal black lines represent the significance level threshold (p adjusted < 0.05). Distribution of platelet contaminants abundances across plasma samples (**D**). Samples are ranked according to their $IDAA_{1C}$ value at 3 months postdiagnosis (i.e., smallest $IDAA_{1C}$ is n°1). The level of significance is represented as 0 (adjusted p value > 0.05) or 1 (adjusted p value < 0.05). The color of the points represents $IDAA_{1C}$ score.

Qualitative and statistical approaches to identify the most reliable candidates

To identify the most promising biomarkers for validation studies, we performed an exhaustive literature search of all potential candidates (i.e., qualitative approach). These were further classified into six pivotal clusters related to diabetes (i.e., insulin secretion, cellular stress, inflammation markers, lipid metabolism, muscle, and diabetes-related complications (i.e., micro- and macrovascular)). Relationships between our protein candidates and pathways, gene regulation networks or protein interactions related to diabetes were investigated using *Pathway Studio* software (http://www.pathwaystudio.com) (48). From our list of candidates, forty-five proteins were associated with key mechanisms implicated in T1D physiopathology (e.g., insulin secretion and resistance, inflammation, endothelial reticulum stress and protein folding, regulation of INS gene).

In a complementary way, we performed an unbiased approach using both random forest (with [RFE_RF] or without [RF] recursive feature elimination) and support vector machine (SVM) (i.e., statistical approach). To avoid over-adjustment of the models, post statistical analyses were solely conducted on differentially expressed proteins ($p<0.05$, n=98). Interestingly, 36 proteins (37%) were selected by at least one post-analysis clustering method with some of them being selected by two (n=6, 6%) or three (n=6, 6%) methods (*data not shown*).

Combined filters from both qualitative and statistical approaches were used to funnel potential candidates to a total of 26 plasmatic proteins.

Characteristics of the latter (i.e., fold change [FC] and p value) are represented according to their diabetes-related cluster in **Fig. 4.**

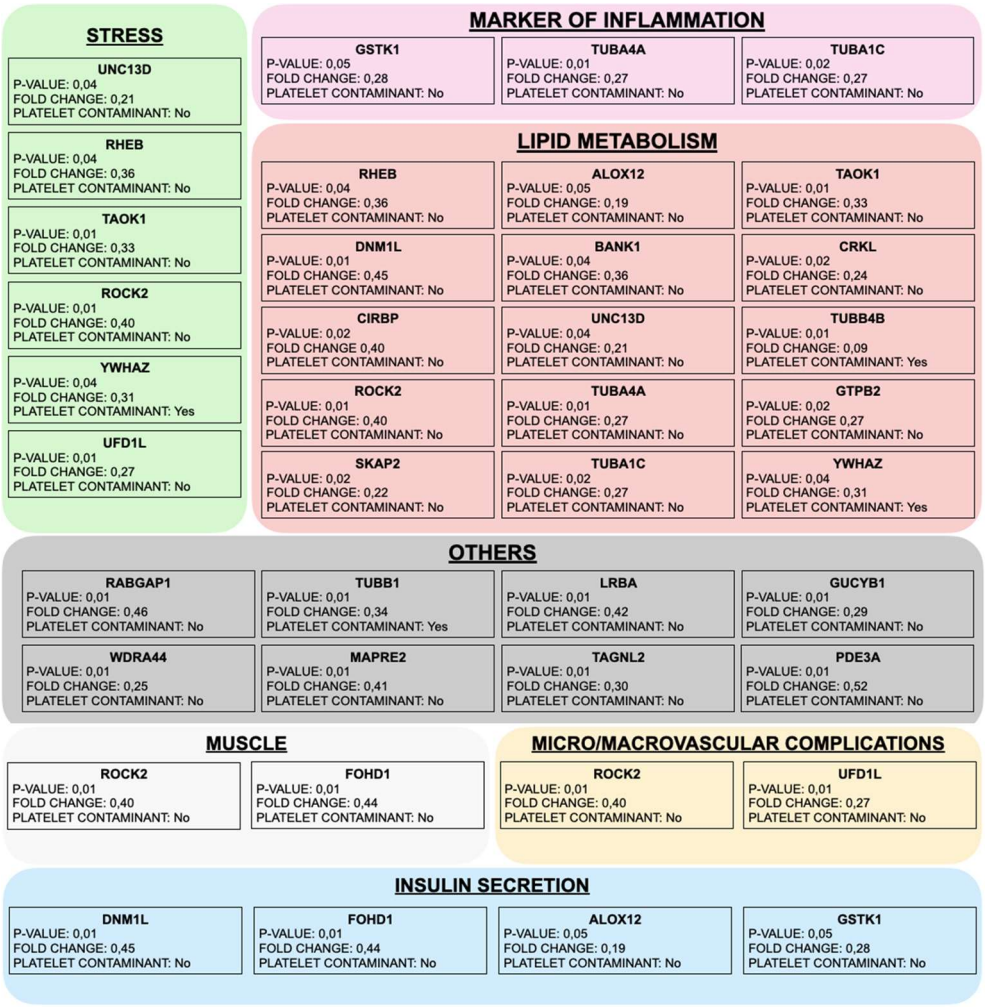

Figure 4: Summary of the 26 protein candidates' characteristics. Protein candidates were classified according to their diabetes-related cluster (i.e., insulin secretion, cellular stress, inflammation, lipotoxicity, vascular complications, or marker of muscle atrophy). Some proteins may be found in more than one cluster. For each protein, adjusted p value, fold change and identification as potential platelet contaminant is indicated. Fold change is expressed in log2 and corresponds to the degree of protein expression change per unit of $IDAA_{1C}$.

Mass spectrometric parallel reaction monitoring validated several protein candidates in raw plasma

To confirm our candidates and ease the translation to clinical settings, we performed targeted PRM proteomic on raw plasma samples of fourteen of the previous patients (i.e., without pretreatment with PM). Quantotypic peptides (i.e., that accurately represent the level of the protein) were identified for 22 out of 26 candidates on SRMAtlas and PeptideAtlas and differed from the ones quantified in the TMT experiment (49) (**Supplemental Table 2**).

Using a single MS run for each sample, we identified and quantified 16 out of the 22 potential candidates. Most of them were quantified in all samples with at least one quantotypic peptide (n=15 [94%]). From these proteins, 13 peptides (11 proteins) significantly correlated with the $IDAA_{1C}$ score ($R = 0.55\text{-}0.86$, $p<0.05$) with peptides from tubulins (i.e., TUBA4A, TUBB1, TUBB4B), YWHAZ and UNC13D showing the strongest correlations (i.e., $R>0.70$) (**Fig. 5**). As $IDAA_{1C}$ values demonstrated a huge gap (i.e., no samples) in the 11-14 range, we performed a subanalysis excluding the patient with the highest $IDAA_{1C}$ (i.e., score of 14). Similar trends were maintained for most of protein candidates with peptides from tubulins (TUBA4A, TUBB1, TUBB4B), YWHAZ and UNC13D significantly correlating with the $IDAA_{1C}$ score ($R>0.6$, $p<0.05$).

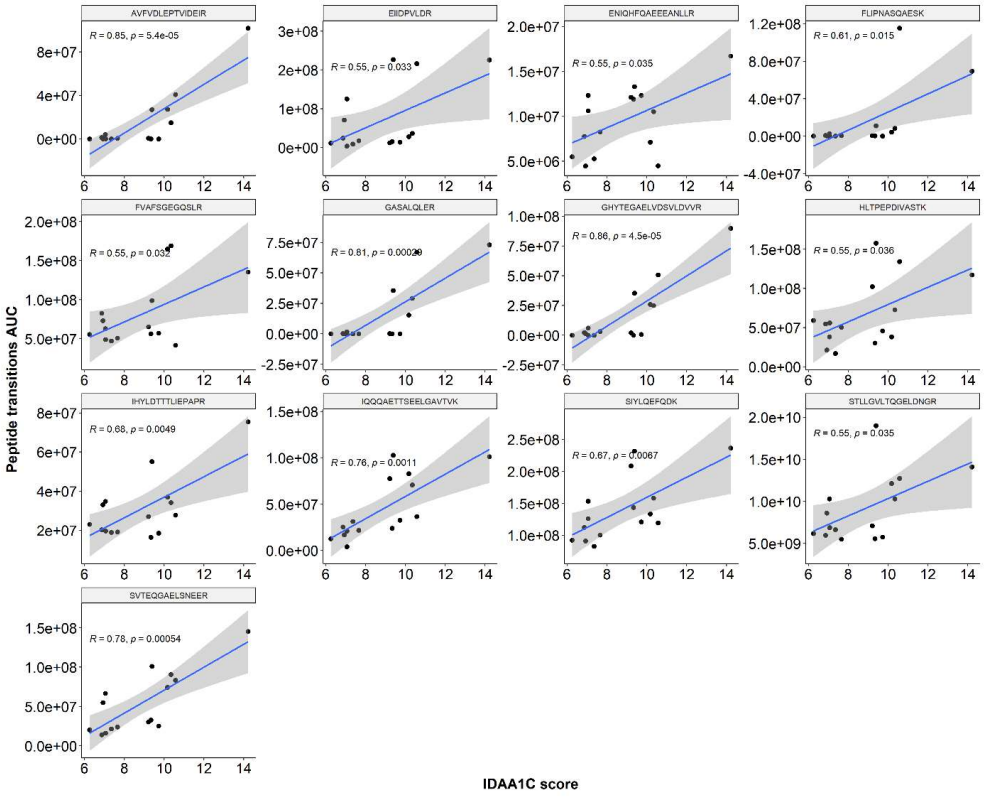

Figure 5: Relations between peptides' plasmatic abundances at clinical onset of type 1 diabetes and $IDAA_{1C}$ score at 3 months postdiagnosis (n=14). AUC were integrated from the MS^2 data for each peptide. Each panel represents linear regression with 95% CI bands (shaded zone) between peptide transitions area under the curve and $IDAA_{1C}$ score. Each point corresponds to one patient. Abbreviations: AUC= area under the curve, $IDAA_{1C}$ = insulin dose-adjusted A1C. Correspondence between quantotypic peptide and protein can be found in **Supplemental Table 2.**

3.7.4 Discussion

Identification of early predictive biomarkers of PR occurrence in patients with new-onset T1D may foster patient-specific interventions (e.g., initiation of immunomodulatory therapies, achieving tight glycemic control) and prevent further β-cell loss. In this context, quantification of hundreds of individual proteins in easily collectible samples may provide potential new biomarkers (e.g., plasma proteome).To the best of our knowledge, we performed the first and most extensive shotgun proteomic analysis to characterize the plasmatic proteome at T1D onset and identify predictive markers of PR. Indeed, our subsidiary analysis allowed the identification and relative quantification of nearly 1500 unique proteins across all sixteen samples. Furthermore, our bottom-up strategy yielded the identification of 98 potential predictive biomarkers of PR that correlated with $IDAA_{1C}$ score at 3 months. A combination of both qualitative (i.e., ORA and Pathway studio) and statistical (i.e., RF and SVM) filters funneled protein candidates to twenty-six which were subsequently classified into six diabetes-related clusters. Finally, several of those candidates were orthogonally validated on raw plasma (i.e., without pre-processing) using single-run targeted proteomic (i.e., PRM method).

Most studies using plasma or serum proteomic to identify new disease biomarkers use a bottom-up strategy (i.e., triangle approach (50)) concomitantly with sample decomplexification methods. Similarly, we performed shotgun proteomic on depleted and fractionated plasma yielding nearly 1700 identified and 1500 quantified proteins across all samples. These results contrasted with other proteomic studies on body fluids (i.e.,

plasma or serum) in the field of T1D that hardly quantified more than a thousand unique proteins (51–55). Interestingly, our approach also allowed the quantification of more than a hundred low-abundance proteins which demonstrated to be a major reservoir for potential biomarkers (46,50). Nonetheless, caution must be taken when interpreting results from depleted matrix as depletion modifies the composition of the initial proteome. Subsequent validation of biomarker candidates in non-depleted samples and using a complementary method is thus mandatory (e.g., PRM, ELISA, western blot) (50).

Another source of confounding factors leading to the paucity of reproducibility in proteomic studies stands in the poor standardization of the type of matrix used (i.e., serum *vs* plasma) and the sample pretreatment steps (i.e., timing before sample pretreatment, centrifugation parameters) (56,57). These elements may lead to heterogeneity in contaminations related to blood cells components (e.g., platelet quantity, hemolysis, coagulation) and alteration of the initial proteome. As suggested by the HUPO project, we used poor-platelet plasma as our matrix (i.e., less influence of coagulation and platelets) (45) and performed a quality-check of the samples using recently described method (41). Unsurprisingly, some of our candidates were classified as quality markers of platelet contamination though a majority of proteins identified in the platelet list did not correlate with $IDAA_{1C}$ score (i.e., n=15/26, 58%). Moreover, some contaminants that were significantly correlated to $IDAA_{1C}$ score demonstrated a role in the diabetes field (e.g., YWHAZ) (**Fig. 4**). Summarizing the influence of these factors on the identification of biomarkers, a recent

study highlighted that more than 54% of plasma proteomic studies reported at least one significantly altered protein that was identified as a potential contaminant (i.e., quality marker) (41).

Interestingly, we identified SKAP2 and Crk-like protein (CRKL) as potential candidates using both TMT labeling and PRM methods ($R>0.67$, $p<0.05$). Both proteins are involved in innate and adaptative immunity respectively, which are dysregulated in patients with T1D (58,59). On the one hand, SKAP2 influenced both β-cell sensitivity to cytokines (i.e., increased sensitivity when downregulated) and macrophage activity (i.e., increased activity when upregulated) (60,61). Furthermore, specific polymorphism of SKAP2 gene (i.e., overexpression in immune cells) was associated with an increased pejoration of glucose homeostasis during the first year of T1D (59). Our results may indicate an increase in macrophage activity in nonremitters. On the other hand, CRKL was associated with the selective migration of effector T cells towards inflammatory organs leading to graft-versus-host disease in mice (62) that was reduced after gene knock-out. In humans, overexpression of CRKL was implicated in the development of autoimmune diseases such as systemic lupus erythematosus (63).

T1D is also characterized by a progressive reduction in β-cell mass and insulin secretion. In our dataset, protein 14-3-3 ζ/δ (YWHAZ) correlated with $IDAA_{1C}$ score at +3 months suggesting that increased circulating levels of YWHAZ predicted higher dysglycemia. YWHAZ is a ubiquitous protein that plays a major role in glucose homeostasis. First, overexpression of YWHAZ inhibited incretin secretion with whole-body 14-3-3-KO mice showing an

increase in GLP-1 and consequently insulin circulating levels (64). Secondly, a recent study highlighted the β-cell specific role of 14-3-3ζ isoform on the first phase of insulin secretion (65). Indeed, the authors found that 14-3-3ζ expression restrained insulin release by inhibiting mitochondrial function (65). Furthermore, they also showed that specific inhibition of 14-3-3ζ in cultured human islets from patients with T2D enhanced insulin secretion, mitochondrial function, and proliferation. While most of these mechanisms were described in models and patients with T2D, utilization of drugs either increasing GLP-1 or inhibiting 14-3-3 proteins led to an improvement of insulin secretion in models of T1D (i.e., NOD mice) (66) or patients with new-onset T1D (67).

Insulin deficiency, which is the main feature of T1D, leads to global metabolic disorders affecting the whole organism (e.g., gluco- and lipotoxicity, acidosis, insulinoresistance, proinflammatory conditions). Tubulins are proteins implicated in the formation of cell microtubules. While implications of those proteins have been mainly described in platelet dysfunctions, cancers and inflammation, there is a lack of evidence on the role of these proteins in T1D. Our results may not be directly derived from β cells but rather indicate a global release of those proteins in the context of global metabolic stress and inflammation.

Finally, targeting a clinically reliable witness of glucose homeostasis, we chose to predict the $IDAA_{1C}$ score rather than residual C-peptide secretion. Recent data arising from our group and others supported strong correlations between $IDAA_{1C}$ and direct measures of glucose homeostasis

(e.g., clinical parameters or CGM data) while poorly correlating with residual β-cell secretion markers (16,68–70). Furthermore, our results also supported that PR period was characterized by a continuum of glucose dysregulations rather than a dichotomic event. Indeed, significant proteins were only identified by linear regression predicting $IDAA_{1C}$ values at 3 months. This hypothesis was previously supported by our team (16) which showed an overlap of glucose homeostasis metrics in remitters and nonremitters with intermediate $IDAA_{1C}$ values (i.e., in the 8-10 range).

Our study demonstrates several strengths. This is the first study that performed a deep characterization of plasma proteome at diagnosis of T1D to identify predictive markers of PR period in a pediatric cohort. Furthermore, we performed a quality check of plasma sample data to identify potential bias linked to blood cell contamination. Finally, protein candidates were validated using a complementary method (i.e., targeted proteomic) that allowed the validation of several candidates in raw plasma using a single MS-run with high sensitivity and specificity, improving the translation of the method to the clinic.

Our study was also limited by different factors. Utilization of low-abundance enrichment (i.e., Proteominer) over high-affinity depletion (e.g., Top14™) methods may lead to a loss of proteins in the unbound fraction. Nonetheless, this loss may be limited as recent study showed on the one hand high overlap between bound and unbound PM fractions (91%) and on the second hand between ProteoMiner and other depletion methods (71). Contaminant proteins belonging to platelets, erythrocytes and coagulation

cascade were detected in all samples leading to potential bias. Nonetheless, the impact of the latter was limited as their distribution demonstrated to be homogeneous across all samples (i.e., analysis and identical SOP across recruiting centers). Also, the patient with an $IDAA_{1C}$ value of 14 acted as an outlier in our analysis. Our results are also limited by the small sample size and should be further validated in a bigger cohort.

3.7.5 Conclusion

Our study deepened the characterization of plasma proteome of children with new-onset T1D. Interestingly, abundance of nearly a hundred unique proteins at disease onset significantly predicted $IDAA_{1C}$ score at 3 months postdiagnosis but not the remission status, supporting PR as a continuum rather than a binary phenomenon. Quantitative (e.g., statistical) and quality (e.g., contaminants or pathways) filters funneled candidate list to twenty-six proteins that were linked to pivotal clusters related to diabetes (i.e., insulin secretion, cellular stress, inflammation markers, lipid metabolism, muscle, and diabetes-related complications (i.e., micro- and macrovascular)). In a translational perspective, twelve of these predictive candidates were validated in the raw plasma using single-run targeted proteomic. We believe that the identification of new predictive biomarkers of PR and β-cell function is key to stratify patients with new-onset T1D for β-cell preservation therapies.

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3.7.6 References

1. DiMeglio LA, Evans-Molina C, Oram RA. Type 1 diabetes. Lancet. 2018 Jun;391(10138):2449–62.

2. Atkinson MA, Roep BO, Posgai A, Wheeler DCS, Peakman M. The challenge of modulating β-cell autoimmunity in type 1 diabetes. Lancet Diabetes Endocrinol. 2019;7(1):52–64.

3. Roep BO, Thomaidou S, van Tienhoven R, Zaldumbide A. Type 1 diabetes mellitus as a disease of the β-cell (do not blame the immune system?). Nat Rev Endocrinol. 2021 Dec 8;17(3):150–61.

4. Mallone R, Eizirik DL. Presumption of innocence for beta cells: why are they vulnerable autoimmune targets in type 1 diabetes? Diabetologia. 2020 Oct 7;63(10):1999–2006.

5. Nathan DM. Realising the long-term promise of insulin therapy: the DCCT/EDIC study. Diabetologia. 2021 May 6;64(5):1049–58.

6. Saiyed M, Hasnani D, Alonso GT, Richmond E, Besançon S, Cotterill A, et al. Worldwide differences in childhood type 1 diabetes: The SWEET experience. Pediatr Diabetes. 2021 Mar 22;22(2):207–14.

7. Rewers M, Pihoker C, Donaghue K, Hanas R, Swift P, Klingensmith GJ. Assessment and monitoring of glycemic control in children and adolescents with diabetes. Pediatr Diabetes. 2009 Sep;10:71–81.

8. Chatwin H, Broadley M, Speight J, Cantrell A, Sutton A, Heller S, et al. The impact of hypoglycaemia on quality of life outcomes among adults with type 1 diabetes: A systematic review. Diabetes Res Clin Pract. 2021;174:108752.

9. Mathieu C. Minimising hypoglycaemia in the real world: the challenge of insulin. Diabetologia. 2021;64(5):978–84.

10. Morgan E, Cardwell CR, Black CJ, McCance DR, Patterson CC. Excess mortality in Type 1 diabetes diagnosed in childhood and adolescence: a systematic review of population-based cohorts. Acta Diabetol. 2015;52(4):801–7.

11. Miller KM, Foster NC, Beck RW, Bergensta RM, DuBose SN, DiMeglio LA, et al. Current state of type 1 diabetes treatment in the U.S.: Updated data from the t1d exchange clinic registry. Diabetes Care. 2015;38(6):971–8.

12. Livingstone SJ, Levin D, Looker HC, Lindsay RS, Wild SH, Joss N, et al.

Estimated life expectancy in a scottish cohort with type 1 diabetes, 2008-2010. JAMA - J Am Med Assoc. 2015;313(1):37–44.

13. Petrie JR, Sattar N. Excess Cardiovascular Risk in Type 1 Diabetes Mellitus. Circulation. 2019 Feb 5;139(6):744–7.

14. Deligne C, You S, Mallone R. Personalized Immunotherapies for Type 1 Diabetes: Who, What, When, and How? J Pers Med. 2022;12(4):542.

15. Linsley PS, Greenbaum CJ, Nepom GT. Uncovering pathways to personalized therapies in type 1 diabetes. Diabetes. 2021 Apr 1;70(4):831–41.

16. Pollé OG, Delfosse A, Martin M, Louis J, Gies I, den Brinker M, et al. Glycemic Variability Patterns Strongly Correlate With Partial Remission Status in Children With Newly Diagnosed Type 1 Diabetes. Diabetes Care. 2022;45(October):2360–8.

17. Battaglia M, Ahmed S, Anderson MS, Atkinson MA, Becker D, Bingley PJ, et al. Introducing the endotype concept to address the challenge of disease heterogeneity in type 1 diabetes. Diabetes Care. 2020;43(1):5–12.

18. Long SA, Thorpe J, DeBerg HA, Gersuk V, Eddy JA, Harris KM, et al. Partial exhaustion of CD8 T cells and clinical response to teplizumab in new-onset type 1 diabetes. Sci Immunol. 2016 Nov 18;1(5).

19. Battaglia M, Buckner JH, Levings MK, Richardson SJ, Wong FS, Tree TI. Identifying the 'Achilles heel' of type 1 diabetes. Clin Exp Immunol. 2021 Apr 23;204(2):167–78.

20. Fonolleda M, Murillo M, Vázquez F, Bel J, Vives-Pi M. Remission Phase in Paediatric Type 1 Diabetes: New Understanding and Emerging Biomarkers. Horm Res Paediatr. 2017;88(5):307–15.

21. Tang R, Zhong T, Wu C, Zhou Z, Li X. The Remission Phase in Type 1 Diabetes: Role of Hyperglycemia Rectification in Immune Modulation. Front Endocrinol (Lausanne). 2019 Dec 3;10.

22. Zhong T, Tang R, Gong S, Li J, Li X, Zhou Z. The remission phase in type 1 diabetes: Changing epidemiology, definitions, and emerging immuno-metabolic mechanisms. Diabetes Metab Res Rev. 2020;36(2):0–2.

23. Bensellam M, Jonas J-C, Laybutt DR. Mechanisms of β-cell dedifferentiation in diabetes: recent findings and future research directions. J Endocrinol. 2018 Feb;236(2):R109–43.

24. Niedzwiecki P, Pilacinski S, Uruska A, Adamska A, Naskret D, Zozulinska-Ziolkiewicz D. Influence of remission and its duration on development of early microvascular complications in young adults with type 1 diabetes. J Diabetes Complications. 2015 Nov;29(8):1105–11.

25. Besser REJ, Bell KJ, Couper JJ, Ziegler A-G, Wherrett DK, Knip M, et al. <scp>ISPAD</scp> Clinical Practice Consensus Guidelines 2022: Stages of type 1 diabetes in children and adolescents. Pediatr Diabetes. 2022 Dec 30;23(8):1175–87.

26. Zhang Q, Fillmore TL, Schepmoes AA, Clauss TRW, Gritsenko MA, Mueller PW, et al. Serum proteomics reveals systemic dysregulation of innate immunity in type 1 diabetes. J Exp Med. 2013;210(1):191–203.

27. Nakayasu ES, Qian WJ, Evans-Molina C, Mirmira RG, Eizirik DL, Metz TO. The role of proteomics in assessing beta-cell dysfunction and death in type 1 diabetes. Expert Rev Proteomics. 2019;16(7):569–82.

28. Liu CW, Bramer L, Webb-Robertson BJ, Waugh K, Rewers MJ, Zhang Q. Temporal expression profiling of plasma proteins reveals oxidative stress in early stages of Type 1 Diabetes progression. J Proteomics. 2018;172(April 2017):100–10.

29. McCafferty C, Chaaban J, Ignjatovic V. Plasma proteomics and the paediatric patient. Expert Rev Proteomics. 2019 May 4;16(5):401–11.

30. Mayer-Davis EJ, Kahkoska AR, Jefferies C, Dabelea D, Balde N, Gong CX, et al. ISPAD Clinical Practice Consensus Guidelines 2018: Definition, epidemiology, and classification of diabetes in children and adolescents. Pediatr Diabetes. 2018;19:7–19.

31. Roberts C. Tanner's Puberty Scale: Exploring the historical entanglements of children, scientific photography and sex. Sexualities. 2016;19(3):328–46.

32. Mortensen HB, Hougaard P, Swift P, Hansen L, Holl RW, Hoey H, et al. New definition for the partial remission period in children and adolescents with type 1 diabetes. Diabetes Care. 2009;32(8):1384–90.

33. Millioni R, Tolin S, Puricelli L, Sbrignadello S, Fadini GP, Tessari P, et al. High abundance proteins depletion vs low abundance proteins enrichment: Comparison of methods to reduce the plasma proteome complexity. PLoS One. 2011;6(5).

34. Lee PY, Osman J, Low TY, Jamal R. Plasma/serum proteomics: depletion strategies for reducing high-abundance proteins for biomarker discovery.

Bioanalysis. 2019 Oct;11(19):1799–812.

35. Paul J, Veenstra TD. Separation of Serum and Plasma Proteins for In-Depth Proteomic Analysis. Separations. 2022 Apr 1;9(4):89.

36. Bradford M. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. Anal Biochem. 1976 May 7;72(1–2):248–54.

37. Ouni E, Ruys SP dit, Dolmans M-M, Herinckx G, Vertommen D, Amorim CA. Divide-and-Conquer Matrisome Protein (DC-MaP) Strategy: An MS-Friendly Approach to Proteomic Matrisome Characterization. Int J Mol Sci. 2020 Nov 30;21(23):9141.

38. Batth TS, Francavilla C, Olsen J V. Off-Line High-pH Reversed-Phase Fractionation for In-Depth Phosphoproteomics. J Proteome Res. 2014 Dec 5;13(12):6176–86.

39. Narváez-Rivas M, Vu N, Chen G-Y, Zhang Q. Off-line mixed-mode liquid chromatography coupled with reversed phase high performance liquid chromatography-high resolution mass spectrometry to improve coverage in lipidomics analysis. Anal Chim Acta. 2017 Feb;954:140–50.

40. Smyth GK. Linear Models and Empirical Bayes Methods for Assessing Differential Expression in Microarray Experiments. Stat Appl Genet Mol Biol. 2004 Jan 12;3(1):1–25.

41. Geyer PE, Voytik E, Treit P V, Doll S, Kleinhempel A, Niu L, et al. Plasma Proteome Profiling to detect and avoid sample-related biases in biomarker studies. EMBO Mol Med. 2019;11(11):e10427.

42. Kuhn M. Package “caret” Title Classification and Regression Training. 2022;

43. Bourmaud A, Gallien S, Domon B. Parallel reaction monitoring using quadrupole-Orbitrap mass spectrometer: Principle and applications. Proteomics. 2016 Aug;16(15–16):2146–59.

44. Wentworth JM, Bediaga NG, Giles LC, Ehlers M, Gitelman SE, Geyer S, et al. Beta cell function in type 1 diabetes determined from clinical and fasting biochemical variables. Diabetologia. 2019;62(1):33–40.

45. Omenn GS. THE HUPO Human Plasma Proteome Project. PROTEOMICS – Clin Appl. 2007 Aug;1(8):769–79.

46. Keshishian H, Burgess MW, Gillette MA, Mertins P, Clauser KR, Mani DR, et al. Multiplexed, Quantitative Workflow for Sensitive Biomarker Discovery

in Plasma Yields Novel Candidates for Early Myocardial Injury. Mol Cell Proteomics. 2015 Sep;14(9):2375–93.

47. Liu C-W, Bramer L, Webb-Robertson B-J, Waugh K, Rewers MJ, Zhang Q. Temporal profiles of plasma proteome during childhood development. J Proteomics. 2017 Jan;152:321–8.

48. Yuryev A, Kotelnikova E, Daraselia N. Ariadne's ChemEffect and Pathway Studio knowledge base. Expert Opin Drug Discov. 2009 Dec 24;4(12):1307–18.

49. Holman SW, Sims PFG, Eyers CE. The use of selected reaction monitoring in quantitative proteomics. Bioanalysis. 2012 Jul;4(14):1763–86.

50. Geyer PE, Holdt LM, Teupser D, Mann M. Revisiting biomarker discovery by plasma proteomics. Mol Syst Biol. 2017;13(9):942.

51. von Toerne C, Laimighofer M, Achenbach P, Beyerlein A, de las Heras Gala T, Krumsiek J, et al. Peptide serum markers in islet autoantibody-positive children. Diabetologia. 2017;60(2):287–95.

52. Albrethsen J, Kaas A, Schönle E, Swift P, Kocova M, Gammeltoft S, et al. Evaluation of a type 1 diabetes serum cohort by SELDI-TOF MS protein profiling. PROTEOMICS - Clin Appl. 2009 Mar;3(3):383–93.

53. Zhi W, Purohit S, Carey C, Wang M, She J-X. Proteomic Technologies for the Discovery of Type 1 Diabetes Biomarkers. J Diabetes Sci Technol. 2010 Jul 1;4(4):993–1002.

54. Zhi W, Sharma A, Purohit S, Miller E, Bode B, Anderson SW, et al. Discovery and Validation of Serum Protein Changes in Type 1 Diabetes Patients Using High Throughput Two Dimensional Liquid Chromatography-Mass Spectrometry and Immunoassays. Mol Cell Proteomics. 2011 Nov;10(11):M111.012203.

55. Moulder R, Bhosale SD, Erkkilä T, Laajala E, Salmi J, Nguyen E V., et al. Serum proteomes distinguish children developing type 1 diabetes in a cohort with HLA-conferred susceptibility. Diabetes. 2015;64(6):2265–78.

56. Halvey P, Farutin V, Koppes L, Gunay NS, Pappas DA, Manning AM, et al. Variable blood processing procedures contribute to plasma proteomic variability. Clin Proteomics. 2021;18(1):1–14.

57. Nambu M, Nishiumi S, Kobayashi T, Masuda T, Ito S, Yoshida M, et al. Effects of differences in pre-analytical processing on blood protein profiles

determined with SWATH-MS. J Proteomics. 2020 Jul;223:103824.

58. Rutsch N, Chamberlain CE, Dixon W, Spector L, Letourneau-Freiberg LR, Lwin WW, et al. Diabetes With Multiple Autoimmune and Inflammatory Conditions Linked to an Activating SKAP2 Mutation. Diabetes Care. 2021 Aug 1;44(8):1816–25.

59. Fløyel T, Meyerovich K, Prause MC, Kaur S, Frørup C, Mortensen HB, et al. SKAP2 , a Candidate Gene for Type 1 Diabetes, Regulates β-Cell Apoptosis and Glycemic Control in Newly Diagnosed Patients. Diabetes. 2021 Feb 1;70(2):464–76.

60. Sano H, Imagawa A. Research following genome-wide association study focuses on the multifaceted nature of Src kinase-associated phosphoprotein 2 in type 1 diabetes. J Diabetes Investig. 2022 Apr 4;13(4):611–3.

61. Brorsson CA, Nielsen LB, Andersen ML, Kaur S, Bergholdt R, Hansen L, et al. Genetic Risk Score Modelling for Disease Progression in New-Onset Type 1 Diabetes Patients: Increased Genetic Load of Islet-Expressed and Cytokine-Regulated Candidate Genes Predicts Poorer Glycemic Control. J Diabetes Res. 2016;2016:1–8.

62. Huang Y, Clarke F, Karimi M, Roy NH, Williamson EK, Okumura M, et al. CRK proteins selectively regulate T cell migration into inflamed tissues. J Clin Invest. 2015 Mar 2;125(3):1019–32.

63. Shi X, Ye L, Xu S, Guo G, Zuo Z, Ye M, et al. Downregulated miR-29a promotes B cell overactivation by upregulating Crk-like protein in systemic lupus erythematosus. Mol Med Rep. 2020 May 21;22(2):841–9.

64. Lim GE, Piske M, Lulo JE, Ramshaw HS, Lopez AF, Johnson JD. Ywhaz/14-3-3ζ Deletion Improves Glucose Tolerance Through a GLP-1-Dependent Mechanism. Endocrinology. 2016 Jul 1;157(7):2649–59.

65. Mugabo Y, Zhao C, Tan JJ, Ghosh A, Campbell SA, Fadzeyeva E, et al. 14-3-3ζ Constrains insulin secretion by regulating mitochondrial function in pancreatic β cells. JCI Insight. 2022 Apr 22;7(8).

66. Maki T, Gottschalk R, Ogawa N, Monaco AP. Prevention and Cure of Autoimmune Diabetes in Nonobese Diabetic Mice by Continuous Administration of FTY720. Transplantation. 2005 May;79(9):1051–5.

67. Ceriello A, Novials A, Ortega E, Canivell S, La Sala L, Pujadas G, et al. Glucagon-Like Peptide 1 Reduces Endothelial Dysfunction, Inflammation,

and Oxidative Stress Induced by Both Hyperglycemia and Hypoglycemia in Type 1 Diabetes. Diabetes Care. 2013 Aug 1;36(8):2346–50.

68. Boyle KD, Keyes-Elstein L, Ehlers MR, McNamara J, Rigby MR, Gitelman SE, et al. Two- and Four-Hour tests differ in capture of C-peptide responses to a mixed meal in type 1 diabetes. Diabetes Care. 2016;39(6):e76–8.

69. Ruan Y, Willemsen RH, Wilinska ME, Tauschmann M, Dunger DB, Hovorka R. Mixed-meal tolerance test to assess residual beta-cell secretion: Beyond the area-under-curve of plasma C-peptide concentration. Pediatr Diabetes. 2019 May 1;20(3):282–5.

70. Carr ALJ, Oram RA, Marren SM, McDonald TJ, Narendran P, Andrews RC. Measurement of Peak C-Peptide at Diagnosis Informs Glycemic Control but not Hypoglycemia in Adults with Type 1 Diabetes. J Endocr Soc. 2021;5(10):1–10.

71. Kaur G, Poljak A, Ali SA, Zhong L, Raftery MJ, Sachdev P. Extending the Depth of Human Plasma Proteome Coverage Using Simple Fractionation Techniques. J Proteome Res. 2021;20(2):1261–79.

3.7.7 Supplemental material

Supplemental Table 1: List of plasma proteins significantly correlating with $IDAA_{1C}$ score.

Protein accession number	Name	GN	logFC	Average Expression	t	adj.P.Val
G8JLD5	Dynamin-1-like protein	DNM1L	0.454	0.683	6.532	0.007
Q9H4B7	Tubulin beta-1 chain	TUBB1	0.342	3.838	6.311	0.007
Q9Y613	FH1/FH2 domain-containing protein 1	FHOD1	0.448	0.600	6.243	0.007
Q6PJW8	Consortin	CNST	0.503	-2.665	6.022	0.007
Q9Y3P9	Rab GTPase-activating protein 1	RABGAP1	0.458	-2.245	6.009	0.007
Q5TCU3	Tropomyosin beta chain	TPM2	0.324	0.736	5.822	0.007
Q5JSH3	WD repeat-containing protein 44	WDR44	0.255	2.019	5.734	0.007
D9YZV8	Tropomyosin 1 (Alpha) isoform 7	TPM1	0.429	3.733	5.700	0.007
Q8N392	Rho GTPase-activating protein 18	ARHGAP18	0.409	0.062	5.640	0.007
Q5JXI8	Four and a half LIM domains 1 (Fragment)	FHL1	0.344	1.063	5.625	0.007
Q13418	Integrin-linked protein kinase	ILK	0.342	3.234	5.498	0.007
Q9HBI1	Beta-parvin	PARVB	0.434	2.524	5.484	0.007
P67936	Tropomyosin alpha-4 chain	TPM4	0.596	2.893	5.454	0.007
H0YAC6	Lipopolysaccharide-responsive and beige-like anchor protein (Fragment)	LRBA	0.424	0.355	5.452	0.007
Q14008	Cytoskeleton-associated protein 5	CKAP5	0.294	-1.659	5.442	0.007
O00151	PDZ and LIM domain protein 1	PDLIM1	0.269	1.399	5.381	0.007
E9PP21	Cysteine and glycine-rich protein 1	CSRP1	0.281	1.361	5.218	0.008
O95810	Serum deprivation-response protein	SDPR	0.440	2.608	5.199	0.008
P09493-3	Isoform 3 of Tropomyosin alpha-1 chain	TPM1	0.491	-1.095	5.191	0.008
H7BXY5	Nexilin (Fragment)	NEXN	0.740	-1.227	5.180	0.008
O00139	Kinesin-like protein KIF2A	KIF2A	0.286	1.598	5.063	0.010
O43182	Rho GTPase-activating protein 6	ARHGAP6	0.404	-0.820	5.005	0.010

E9PCN2	Guanylate cyclase soluble subunit beta-1	GUCY1B3	0.293	-0.335	4.976	0.010
P18206	Vinculin	VCL	0.357	4.777	4.974	0.010
A8MUB1	Tubulin alpha-4A chain	TUBA4A	0.514	2.152	4.961	0.010
Q14432	cGMP-inhibited 3',5'-cyclic phosphodiesterase A	PDE3A	0.520	-2.081	4.949	0.010
Q15404	Ras suppressor protein 1	RSU1	0.414	2.731	4.940	0.010
Q86UX7	Fermitin family homolog 3	FERMT3	0.396	3.825	4.855	0.011
Q7LDG7	RAS guanyl-releasing protein 2	RASGRP2	0.322	1.427	4.818	0.012
Q9Y490	Talin-1	TLN1	0.457	6.048	4.810	0.012
P68371	Tubulin beta-4B chain	TUBB4B	0.289	2.789	4.730	0.013
Q92890-1	Isoform Long of Ubiquitin fusion degradation protein 1 homolog	UFD1L	0.267	-2.202	4.721	0.013
G5E9I6	Microtubule-associated protein RP/EB family member 2	MAPRE2	0.409	1.407	4.718	0.013
P67936-2	Isoform 2 of Tropomyosin alpha-4 chain	TPM4	0.530	4.066	4.690	0.013
C9JK39	cAMP-dependent protein kinase catalytic subunit beta	PRKACB	0.239	0.050	4.642	0.014
Q86TP1	Protein prune homolog	PRUNE	0.291	1.543	4.595	0.015
O75116	Rho-associated protein kinase 2	ROCK2	0.396	0.772	4.557	0.015
Q9NZN3	EH domain-containing protein 3	EHD3	0.341	1.923	4.551	0.015
F8VW92	Tubulin beta chain	TUBB	0.307	2.020	4.547	0.015
Q5SW96	Low density lipoprotein receptor adapter protein 1	LDLRAP1	0.482	-3.174	4.532	0.015
Q7L7X3	Serine/threonine-protein kinase TAO1	TAOK1	0.329	-1.195	4.529	0.015
F5H1F6	Vacuolar protein sorting-associated protein 37B (Fragment)	VPS37B	0.218	-2.935	4.528	0.015
Q01813	6-phosphofructokinase type C	PFKP	0.311	2.014	4.521	0.015
P08567	Pleckstrin	PLEK	0.466	1.476	4.484	0.015
Q8NBF2	NHL repeat-containing protein 2	NHLRC2	0.312	-0.323	4.478	0.015
P48059	LIM and senescent cell antigen-like-containing domain protein 1	LIMS1	0.536	1.212	4.449	0.016
C9J2C0	Tubulin alpha-8 chain (Fragment)	TUBA8	0.255	0.904	4.432	0.016
F6RFD5	Destrin	DSTN	0.278	-0.919	4.418	0.016
Q13576	Ras GTPase-activating-like protein IQGAP2	IQGAP2	0.252	3.114	4.409	0.016

F5H5D3	Tubulin alpha-1C chain	TUBA1C	0.490	4.257	4.397	0.016
Q9Y6E0	Serine/threonine-protein kinase 24	STK24	0.282	1.498	4.382	0.016
D6W5Y5	Cold inducible RNA binding protein, isoform CRA_c	CIRBP	0.399	-3.421	4.366	0.016
P37802	Transgelin-2	TAGLN2	0.305	1.095	4.357	0.016
Q9BX10	GTP-binding protein 2	GTPBP2	0.273	-1.921	4.328	0.017
Q9BR76	Coronin-1B	CORO1B	0.232	-1.726	4.305	0.017
Q9H4M9	EH domain-containing protein 1	EHD1	0.331	3.464	4.302	0.017
P24844	Myosin regulatory light polypeptide 9	MYL9	0.429	-0.641	4.269	0.018
P46109	Crk-like protein	CRKL	0.242	-3.250	4.260	0.018
Q9UDY2	Tight junction protein ZO-2	TJP2	0.273	-1.659	4.242	0.019
C9JRJ5	LIM domain-containing protein 1	LIMD1	0.538	-4.869	4.185	0.020
O75563	Src kinase-associated phosphoprotein 2	SKAP2	0.218	-1.077	4.183	0.020
H7BYY1	Tropomyosin 1 (Alpha), isoform CRA_m	TPM1	0.353	-3.190	4.137	0.022
Q8TF42	Ubiquitin-associated and SH3 domain-containing protein B	UBASH3B	0.225	0.245	4.124	0.022
Q15746	Myosin light chain kinase, smooth muscle	MYLK	0.400	0.594	4.124	0.022
B4DDF4	Calponin-2	CNN2	0.300	1.651	4.017	0.027
P01767	Ig heavy chain V-III region BUT	#N/A	0.241	1.214	3.964	0.030
Q59G71	Tensin variant (Fragment)	TNS1	0.191	-1.868	3.947	0.030
Q14247	Src substrate cortactin	CTTN	0.249	1.716	3.928	0.031
F8VV59	Nucleosome assembly protein 1-like 1	NAP1L1	0.443	0.842	3.916	0.031
P47972	Neuronal pentraxin-2	NPTX2	-0.159	-0.767	-3.853	0.035
H3BNZ1	Pyridoxal-dependent decarboxylase domain-containing protein 1	PDXDC1	0.393	-2.323	3.847	0.035
Q6VUC0	Transcription factor AP-2-epsilon	TFAP2E	-0.388	0.344	-3.825	0.035
P07384	Calpain-1 catalytic subunit	CAPN1	0.186	2.164	3.824	0.035
Q27J81	Inverted formin-2	INF2	0.197	0.347	3.809	0.035
P48444	Coatomer subunit delta	ARCN1	0.237	-0.293	3.807	0.035
P41226	Ubiquitin-like modifier-activating enzyme 7	UBA7	0.208	1.190	3.781	0.035
Q14141	Septin-6	SEPT6	0.255	-1.776	3.777	0.035
Q70J99	Protein unc-13 homolog D	UNC13D	0.206	1.235	3.775	0.035

Q86YL5	Testis development-related protein	TDRP	0.288	-2.566	3.775	0.035
Q14644	Ras GTPase-activating protein 3	RASA3	0.264	-3.485	3.772	0.035
Q8NDB2	B-cell scaffold protein with ankyrin repeats	BANK1	0.387	-2.645	3.771	0.035
C9J931	GTP-binding protein Rheb	RHEB	0.362	-0.052	3.769	0.035
P26641	Elongation factor 1-gamma	EEF1G	0.271	1.203	3.766	0.035
Q9H5N1	Rab GTPase-binding effector protein 2	RABEP2	0.278	-4.122	3.749	0.036
Q8NEU8	DCC-interacting protein 13-beta	APPL2	0.249	-1.504	3.744	0.036
F5H0W4	Bridging integrator 2	BIN2	0.400	2.257	3.731	0.037
P63104	14-3-3 protein zeta/delta	YWHAZ	0.310	3.139	3.688	0.039
Q01433	AMP deaminase 2	AMPD2	0.388	0.274	3.687	0.039
Q99961	Endophilin-A2	SH3GL1	0.261	-2.714	3.661	0.041
Q96HC4	PDZ and LIM domain protein 5	PDLIM5	0.206	-2.867	3.637	0.043
B4E0K5	Mitogen-activated protein kinase 14	MAPK14	0.168	-2.630	3.622	0.043
E9PFN5	Glutathione S-transferase kappa 1	GSTK1	0.275	-4.163	3.579	0.047
B1B0M1	GRIP1-associated protein 1	GRIPAP1	0.219	-1.321	3.565	0.048
Q02108	Guanylate cyclase soluble subunit alpha-3	GUCY1A3	0.154	-0.473	3.543	0.050
P18054	Arachidonate 12-lipoxygenase, 12S-type	ALOX12	0.191	1.100	3.536	0.050
Q7L576	Cytoplasmic FMR1-interacting protein 1	CYFIP1	0.225	-1.055	3.533	0.050
P61163	Alpha-centractin	ACTR1A	0.202	-0.443	3.525	0.050
F5H865	TRAF2 and NCK-interacting protein kinase	TNIK	0.171	1.429	3.521	0.050

Abbreviations: FC = fold-change, GN = gene name, adj.P.Val = adjusted p-value.

Supplemental Table 2: List of quantotypic peptides selected for validation in raw plasma using Parrallel Reaction Monitoring (PRM) method.

Compound	Protein	Uniprot accession	m/z	z	Correlation with $IDAA_{1C}$
VISYEEGK	RHEB	Q15382	462.74	2	
ALAESWNAAFLESSAK	RHEB		847.92	2	
ENQTAVDVFR	RHEB		589.79	2	
ALQGASQIIAEIR	DNM1L	O00429	685.39	2	
IIQHC[160]SNYSTQELLR	DNM1L		621.31	3	
SSLLDDLLTESEDMAQR	DNM1L		641.639	3	
SGGYGGSR	CIRBP	Q14011	370.67	2	
SQSGGYSDR	CIRBP		478.71	2	
GFGFVTFENIDDAK	CIRBP		780.37	2	
EFINLQSALESER	ROCK2	O75116	769.39	2	
TLTSDVANLANEK	ROCK2		688.36	2	
THGSEIINDLQGR	ROCK2		480.58	3	
FVAFSGEGQSLR	UFD1L	Q92890	649.33	2	Yes
FQPQSPDFLDITNPK	UFD1L		873.94	2	
AALLNFDEFAVSK	FHOD1	Q9Y613	712.88	2	
VDFEQLTENLGQLER	FHOD1		597.64	3	
HLGTAGTDVDLR	FHOD1		418.89	3	
SIYLQEFQDK	SKAP2	O75563	635.82	2	Yes
GEFAIDGYSVR	SKAP2		607.3	2	
DAEEWVQQLK	SKAP2		623.31	2	
NEDITEPQSILAAAEK	GSTK1	Q9Y2Q3	864.94	2	
GLGSPDPAPC[160]GPAPPGNTK	BANK1	Q8NDB2	597.29	3	
LLILSNSLLR	BANK1		571.37	2	
ALEFPAGSVHVNVYC[160]DGIVK	BANK1		725.7	3	
IQQQAETTSEELGAVTVK	UNC13D	Q70J99	644.67	3	Yes
LPAQLAWEALEQR	UNC13D		508.94	3	
ALLYEDALYTVLHR	UNC13D		559.64	3	
AVFVDLEPTVIDEIR	TUBA4A	P68366	858.46	2	Yes
EIIDPVLDR	TUBA4A		535.3	2	Yes
DVNAAIAAIK	TUBA4A		493.29	2	

DYEEVGADSADGEDEGEEY	TUBA1C	Q9BQE3	1039.88	2	
PTYTNLNR	TUBA1C		489.75	2	
DPEIAELFFK	TAOK1	Q7L7X3	604.81	2	
HNLEQDLVR	TAOK1		375.2	3	
ENIQHFQAEEEANLLR	TAOK1		647.65	3	Yes
IGDQEFDHLPALLEFYK	CRKL	P46109	679.01	3	
TLYDFPGNDAEDLPFK	CRKL		921.43	2	
IHYLDTTTLIEPAPR	CRKL		580.65	3	Yes
GHYTEGAELVDSVLDVVR	TUBB4B	P68371	653.67	3	Yes
SGPFGQIFR	TUBB4B		504.77	2	
INVYYNEATGGK	TUBB4B		664.82	2	
DTGGDGQDSLYK	RABGAP1	P20340	628.28	2	
DDLLLTDFEGALK	RABGAP1		725.38	2	
ILETWGELLSK	RABGAP1		644.86	2	
YFLSGSLDGK	WDR44	Q5JSH3	543.78	2	
ASFSHDFTYLVSGSEDK	WDR44		630.62	3	
HLTPEPDIVASTK	WDR44		469.92	3	Yes
SHHANSPTAGAAK	MAPRE2	Q15555	416.87	3	
FQDNLDFIQWFK	MAPRE2		800.89	2	
EIELLC[160]QEHGQENDDLVQR	MAPRE2		775.7	3	
AFPFHIIFDR	GUCY1B3	Q02153	421.56	3	
IN[115]VSEYTYR	GUCY1B3		573.28	2	
EAQLDEEGQFLVR	GUCY1B3		768.38	2	
GASALQLER	TUBB1	Q9H4B7	472.76	2	Yes
GHYTEGAELIENVLEVVR	TUBB1		676.69	3	
EVDQQLLSVQTR	TUBB1		708.38	2	
VGADITVLR	GTPBP2	Q9BX10	472.28	2	
VFLNILPPLTNSK	GTPBP2		485.96	3	
STLLGVLTQGELDNGR	GTPBP2		558.3	3	Yes
FLIPNASQAESK	YWHAZ	P63104	652.85	2	Yes
SVTEQGAELSNEER	YWHAZ		774.86	2	Yes
TAFDEAIAELDTLSEESYK	YWHAZ		1066.5	2	
AFIQLWAFDAVK	AMBP	P02760	704.88	2	
ETLLQDFR	AMBP		511.27	2	
VVAQGVGIPEDSIFTMADR	AMBP		1003	2	

3.8 Paper V: Influence of the Occurrence and Duration of Partial Remission On Short-term Metabolic Control in Type 1 Diabetes: the DIABHONEY Pediatric Study

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Keywords: type 1 diabetes, partial remission, duration, predictive factors, glycemic variability, CGM, glucose homeostasis

3.8.1 Abstract

Objective: To evaluate the residual effect of partial remission (PR) on immediate post-PR glycemic control according to its occurrence and duration in a cohort of children with type 1 diabetes mellitus (T1DM).

Patients and Methods: Values of glycemic control parameters (i.e., HbA_{1C}, insulin dose-adjusted Hemoglobin A_{1C} [$IDAA_{1C}$], glycemic target-adjusted HbA_{1C} [$GTAA_{1C}$]) and data from continuous glucose monitoring from 189 pediatric patients with new-onset type 1 diabetes were collected retrospectively from 24 months. Patients were characterized according to their remission status (PR^+ and PR^-). PR^+ patients were subdivided into three subgroups regarding PR duration (i.e., short [≥3-≤6 months], intermediate [>6-≤12 months] and long PR [>12-≤14 months]). We compared glycemic control data from each PR^+ subgroup at +6 and +12 months post-PR to PR^- patients at the same post-diagnosis time. Secondly, PR^+ subgroups were compared with each other.

Results: PR^+ patients showed improved glycemic control (i.e., HbA_{1C}, $IDAA_{1C}$ and $GTAA_{1C}$) at +6 months post-PR when compared to non-remitters (PR^-), independently of the PR duration subgroups ($p<0.05$). Interestingly, patients in long PR^+ subgroup exhibited higher positive residual effect than short PR^+ subgroup with lower $GTAA_{1C}$ scores ($p=0.02$), better time in range [TIR] ($p=0.003$), less time in hypoglycemia (10.45% *vs* 16.13%, [$p=0.03$]) and less glycemic variability (83 mg/dL *vs* 99 mg/dL, [$p=0.03$]). No significant differences were found for glucose control between PR^+ and PR^- patients at +12 months post-PR.

Conclusion: This study supports the positive impact of PR occurrence and duration on short-term metabolic control (better HbA_{1C} levels, $IDAA_{1C}$ and $GTAA_{1C}$ scores, TIR and less glycemic variability) with the residual effect increasing according to PR duration.

3.8.2 Introduction

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia resulting from dysfunction in insulin secretion, insulin action, or both. Type 1 diabetes mellitus (T1DM) results from irreversible immune-mediated destruction of pancreatic insulin-producing β cells (1,2). At the time of T1DM diagnosis (usually corresponding to the onset of insulinopenic symptoms), islet β-cell mass is reduced to 10-30%(1,3). Shortly after the initiation of insulin therapy, about 60% of patients with T1DM experience a "partial remission" (PR) period also called "honeymoon period" (4,5).

PR definition has evolved over the years and is still debated (6). In 2009, based on the European Hvidøre Study Group, Mortensen and colleagues (7) suggested identifying PR using the insulin dose-adjusted Hemoglobin A_{1C} ($IDAA_{1C}$) score, readily usable in clinics and integrating both HbA_{1C} levels and daily insulin requirements. The $IDAA_{1C}$ score has now been validated in large pediatric patient cohorts (8–11) and by the International Society for Pediatric and Adolescent Diabetes (ISPAD) (5). It is currently considered as the most recognized standard to define PR when its value is ≤ 9. More recently, the glycemic target-adjusted HbA_{1C} ($GTAA_{1C}$) score has been suggested by our team as an alternative definition of PR that does not depend on insulin requirements and which relies on objective markers of glycemic homeostasis (HbA_{1C} and percentage of normoglycemia). $GTAA_{1C}$ score predicts PR when its value is ≤ 4.5 and is strongly correlated with $IDAA_{1C}$ score (12).

Mechanisms underlying PR remain controversial. A state of improved insulin sensitivity, decreased glucotoxicity, relative escape of β cells from the immune system (by decrease of HLA type I expression) and partial recovery of previously exhausted β-cell function, are key metabolic aspects involved in the remission period (4,13). While a dichotomy prevails in the occurrence or absence of PR, this period is marked by heterogeneity in intensity and duration that may be short, intermediate or long and which is influenced by an array of well-described clinical factors (e.g., age, gender, ketoacidosis at diagnosis) (4,9,14–22).

Subsidiary studies of the Diabetes Control and Complication Trial and the Epidemiology of Diabetes Interventions and Complications (EDIC) trial have highlighted the importance of early optimal glycemic control to prevent micro- and macro-vascular complications of T1DM (23,24). The PR period is key in the early management of T1DM but also as a target period for strategies aiming at preserving endogenous β-cell mass. However, apart from being potential leverage for therapeutic applications, little is known about whether the amplitude (i.e., intensity and duration) of PR influences short-term glycemic control in patients with T1D.

The intuitive clinical experience suggests that the level of glycemic control achieved during PR is difficult to maintain once remission ends - often abruptly, and the ensuing imbalance may become chronic. Furthermore, for some patients, PR appears to play a negative effect: the longer the PR, the greater the glycemic imbalance at the end of remission. Very few studies have evaluated the post-PR period and the underlying risks of diabetes-

related complications (25). Especially, correlation between PR duration and post-remission glycemic control was not extensively studied so far, especially in children. Our DIABHONEY study aims to assess the impact of the occurrence and duration of PR on metabolic control in pediatric patients with T1DM in the immediate post-PR period (i.e., 12 months post-PR).

3.8.3 Material and Methods

Study design and participants

We retrieved from our patient database a retrospective cohort of 398 children and adolescents diagnosed with type 1 diabetes between January 1997 and December 2018 and followed up in the pediatric diabetes clinic of our tertiary health care center (Cliniques universitaires Saint-Luc, Brussels). The study was approved by the local ethical committee (reference CHE:11/JUI/274) and conducted in accordance with the Declaration of Helsinki. Patients eligible were aged between 1 and 18 years old and were diagnosed with new-onset T1DM. T1DM was established according to International Society for Pediatric and Adolescent Diabetes (ISPAD) guidelines (3), based on symptoms of insulinopenia, elevated blood glucose (BG), positive anti-islet autoantibodies (i.e., GAD65, IA2 and insulin), and lack of family history of genetic diabetes. Exclusion criteria were diabetes onset before the age of 1 year, presence of severe chronic medical conditions before the diagnosis of type 1 diabetes (i.e., autoimmune diseases other than type 1 diabetes, active cancer, kidney, liver or adrenal insufficiency) and use of medication that may affect insulin secretion and/or glucose homeostasis (i.e., corticosteroids, sulfonylurea, incretins, diazoxide,

somatostatin, immunomodulatory drugs). Patients with a PR less than 3 months, above 15 months or ongoing at the time of study were also excluded. All patients performed carbohydrate counting and underwent similar dietary education at diagnosis.

Medical records of each patient were reviewed to collect demographic data at diagnosis (i.e., age, gender, date of diagnosis, height, weight, body mass index) as well as quarterly follow-up data until 24 months. This included routine clinical and biological parameters (HbA_{1C} levels [%], insulin doses in total daily dose in IU and IU/kg body weight, $IDAA_{1C}$ and $GTAA_{1C}$ scores, number of severe hypoglycemia) and data from glucose monitoring devices (either using continuous glucose monitoring [CGM] or self-monitoring of blood glucose [SMBG]). The parameters retrieved from CGM or blood glucose meter were: average glucose (mg/dL), glucose variability (glycemic SD [mg/dL], coefficient of variation of glucose [CV, %]), number of glucose measurements, time spent in hypoglycemia (below 70 mg/dL, % total time), number of severe hypoglycemia, time spent in hyperglycemia (above 180 mg/dL, % total time), and time spent in normoglycemia (70-180 mg/dL; % total time) also called time in range (TIR). Body Mass Index (BMI) was calculated using the formula = body weight (kg)/(height (m))^2. Z-scores for height and BMI were assessed using Belgian Flemish reference charts (26). Severe hypoglycemia was defined as an alteration of consciousness (with or without coma or convulsion) requiring external assistance from a tier person to actively administer carbohydrates, intramuscular glucagon or other corrective measures, as described by ISPAD (27).

Partial remission definition and groups

PR was defined by a combination of both $IDAA_{1C}$ and $GTAA_{1C}$ scores below their respective threshold (i.e., $IDAA_{1C} \leq 9$ and $GTAA_{1C} \leq 4.5$). IDAA1C was calculated according to Mortensen and colleagues (7), as such: HbA_{1C} (%) + [4 × insulin dose (U/kg/day)]. The $GTAA_{1C}$ (12) corresponds to: HbA_{1C} (%) – [3 x % of normoglycemic values (70-180 mg/dL)]. The end of PR period was defined as the first follow-up consultation where the patient exhibited both an $IDAA_{1C} > 9$ and a $GTAA_{1C} > 4.5$.

Patients were divided into two groups depending on the occurrence (i.e., positive cohort, PR+ group) or absence of PR (i.e., control or negative cohort, PR- group) at three months post-diagnosis . PR+ group was further divided in three subgroups according to their PR duration: short (PR duration ≥3 and ≤6 months), intermediate (PR duration >6 and ≤12 months) or long PR (PR duration >12 and ≤14 months). Patients with a PR period longer than 14 months were excluded as some might have been misdiagnosed with T1DM (e.g., presenting features of monogenic diabetes) (**Fig. 1**). The control group (i.e., PR-) was determined as patients with $IDAA_{1C}$ (> 9) and $GTAA_{1C}$ (> 4.5) indexes.

Firstly, the positive (PR+) and negative (PR-) cohorts were compared for age, gender and BMI. Secondly, we compared clinical and glucose homeostasis data (listed above) from each of the three subgroups of PR+ patients (classified as having short, intermediate, or long PR) at +6 and +12 months after the end of their PR period, to PR- patients at the same time points after diagnosis (see **Supplemental Fig. S1**). Finally, the follow-up data for children

with short PR (>3 and ≤6 months) were compared to children with intermediate (>6 and ≤12 months) and long PR (>12 and ≤14 months).

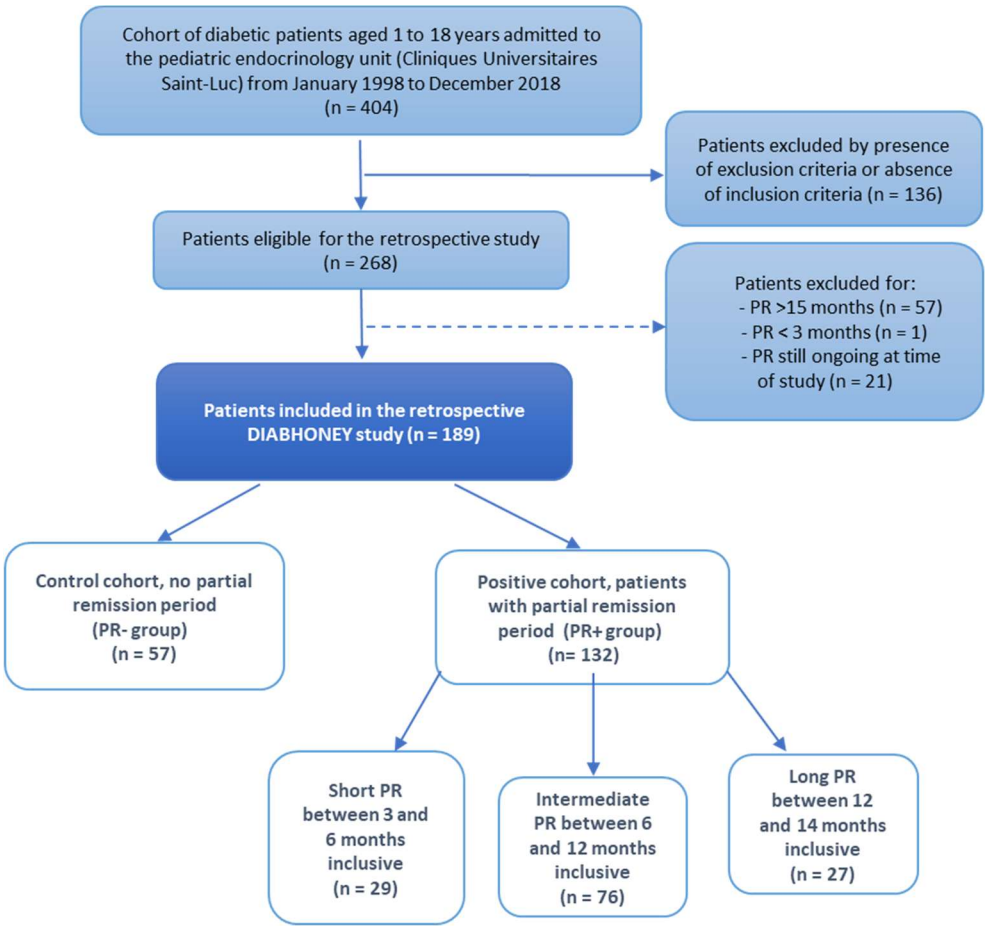

Figure 1: Flowchart of patient groups in the DIABHONEY study.

Statistical analysis

Data were analyzed using the JMP Pro 14.3.0 software. The values of categorical variables are expressed in absolute numbers (n) and relative frequencies (percentage of corresponding total number). The continuous values are expressed as means ± standard deviations. Comparisons between

groups were performed using Student t-test, chi-square test or their non-parametric equivalent (respectively Mann-Whitney U test, Fisher's exact test) as appropriate. A p-value of <0.05 was considered statistically significant.

3.8.4 Results

Characteristics of study participants

A total of 189 patients were included in the DIABHONEY study (**Fig. 1**). PR occurred in 69.8% of the patients (132/189). Mean age at diagnosis was 9.0 ± 3.7 years. Patients in the PR+ cohort were statistically older than children in the PR- cohort (9.6 years vs 7.6 years, respectively; [p=0.001]). The proportion of girls and boys was comparable among the entire cohort (50.3% vs 49.7%, respectively: [p=0.64]), independently of PR status. The mean BMI Z-score at diagnosis was 0.3 ± 1.1, with no difference observed between the two groups (p=0.66) (**Table 1**). Patients undergoing PR were distributed across three subgroups with a majority experiencing intermediate PR (n=76) and a minority experiencing either short or long PR (respectively n=29 and n=27). Overall mean PR duration was 8.7 ± 3.2 months. Most patients were using SMBG (85%) and followed a multiple daily injections insulin regimen (86% vs 14% using insulin pump).

Short PR improves glycemic control ($IDAA_{1C}$, $GTAA_{1C}$) 6 months after remission

To assess the influence of short PR (i.e., >3 - ≤6 months) on T1DM control at 6 and 12 months post-remission, patients experiencing short PR were compared to PR- controls at the same post-diagnosis time points (**Fig. 2**, **Supplemental Fig. S1**). Six months after the end of short PR, HbA_{1C} levels were significantly lower in PR+ children compared to PR- patients (p=0.03). Similarly, $IDAA_{1C}$ and $GTAA_{1C}$ scores were lower in PR+ cohort when compared to the control group (p=0.008 and p=0.02, respectively). No other differences were observed between both groups for other clinical or glycemic control parameters, including the occurrence of severe hypoglycemia events and the number of daily glycemic tests. At 12 months after the end of short PR, there were no significant differences in the metabolic follow-up data (**Fig. 2**).

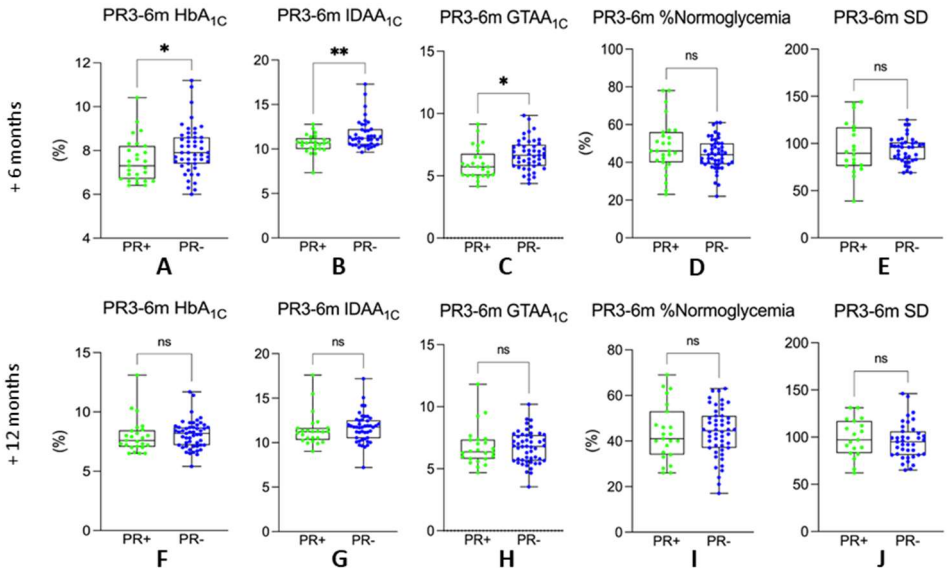

Figure 2: Assessment of the influence of short PR (>3-≤6 months) at 6- and 12-months post-remission. Comparison of the short PR^+ cohort to the control PR^- cohort, matched at the same post-diagnosis time for different parameters. Comparison of short PR^+ cohort at 6 months post-PR *vs* PR^- cohort at 12 months (A): for HbA_{1C}, (B): for $IDAA_{1C}$, (C): for $GTAA_{1C}$, (D): for TIR, (E): for glycemic variability. Comparison of short PR^+ cohort at 12 months post-PR *vs* PR^- cohort at 18 months (F): for HbA_{1C}, (G): for $IDAA_{1C}$, (H): for $GTAA_{1C}$, (I): for TIR, (J): for glycemic variability. Box plots display the median, 25th and 75th percentiles, and range the different parameters between PR^+ group (green points) and PR^- group (blue points). Levels of significance are represented as follows: ns ($p>0.05$), * ($p<0.05$), ** ($p<0.01$), *** ($p<0.001$). PR: partial remission. HbA_{1C}: hemoglobin A1c. $IDAA_{1C}$: insulin dose-adjusted HbA_{1C}. $GTAA_{1C}$: glycemic target-adjusted HbA_{1C}. TIR: time in range. SD: standard deviation.

Intermediate PR significantly improves glycemic control (HbA_{1C}, $IDAA_{1C}$, $GTAA_{1C}$, TIR) 6 months after remission

To assess the influence of intermediate PR (i.e., >6 - ≤12 months) on T1DM control at 6 and 12 months post-remission, intermediate PR+ patients were compared with those in the matched PR- cohort at the same post-diagnosis time points (**Fig. 3**, **Supplemental Fig. S1**). As compared to results from short PR group, patients experiencing intermediate PR exhibited significative differences in their glycemic control 6 months after remission when compared to PR- group: HbA_{1C} levels were significantly lower in PR+ group compared to the PR- control group ($p=0.04$), as were $IDAA_{1C}$ and $GTAA_{1C}$ scores ($p=0.02$ and $p=0.01$ respectively). The TIR was significantly higher in PR+ patients (42 ± 10 % vs 50 ± 15 %, [$p<0.001$]) with less glycemic variability ($p=0.012$) (**Fig. 3**). Mean blood glucose was also statistically better in the PR+ group compared to the control PR- group (164 ± 35 mg/dL vs 175 ± 29 mg/dL, [$p=0.04$]). No other differences were observed between both groups for other clinical or glycemic control parameters, including the occurrence of

severe hypoglycemia events and the number of daily glycemic tests. At 12 months after the end of intermediate PR, we found no significant difference in any of the studied data, except for $IDAA_{1C}$, significantly lower in PR+ patients (p=0.013). Comparing both CGM and SMBG values demonstrate similar results for glucose homeostasis parameters (all p-values > 0.05, *data not shown*).

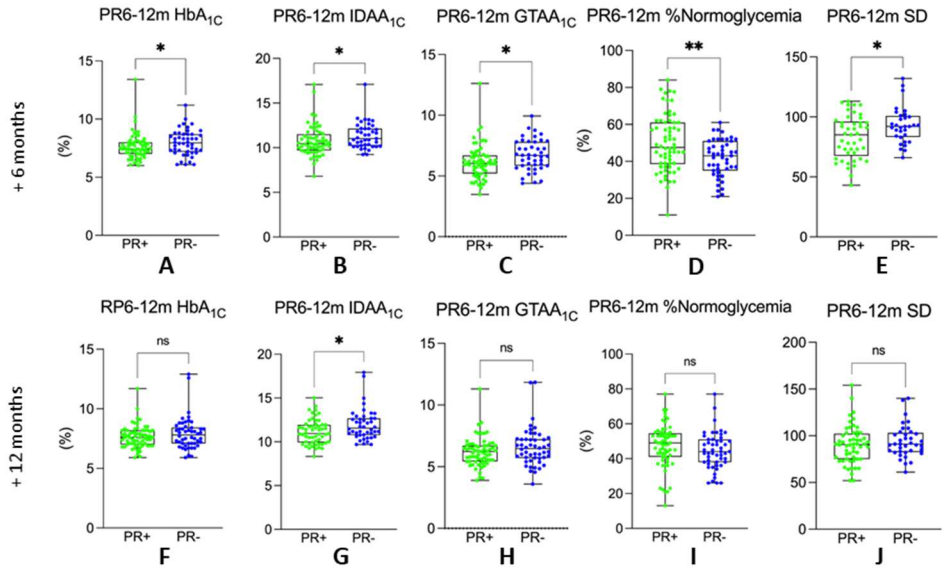

Figure 3: Assessment of the influence of intermediate PR (>6-≤12 months) at 6- and 12-months post-remission. Comparison of the intermediate PR^+ cohort to the control PR^- cohort, matched at the same post-diagnosis time for different parameters. Comparison of intermediate PR^+ cohort at 6 months post-PR *vs* PR^- cohort at 15 months (A): for HbA_{1C}, (B): for $IDAA_{1C}$, (C): for $GTAA_{1C}$, (D): for TIR, (E): for glycemic variability. Comparison of intermediate PR^+ cohort at 12 months post-PR *vs* PR^- cohort at 21 months (F): for HbA_{1C}, (G): for $IDAA_{1C}$, (H): for $GTAA_{1C}$, (I): for TIR, (J): for glycemic variability. Box plots display the median, 25th and 75th percentiles, and range the different parameters between PR^+ group (green points) and PR^- group (blue points). Levels of significance are represented as follows: ns (p>0.05), * (p<0.05), ** (p<0.01), *** (p<0.001). PR: partial remission. HbA_{1C}: hemoglobin A1c. $IDAA_{1C}$: insulin dose-adjusted HbA_{1C}. $GTAA_{1C}$: glycemic target-adjusted HbA_{1C}. TIR: time in range. SD: standard deviation.

Long PR improves glycemic control (HbA_{1C}, $IDAA_{1C}$, $GTAA_{1C}$, TIR) and glycemic variability 6 months after remission

To assess the influence of long remission (i.e., >12 - ≤14 months) on T1DM control at 6 and 12 months post-remission, long PR patients were compared with those in the PR- control group at the same post-diagnosis time points (**Fig. 4**, **Supplemental Fig. S1**). As observed previously for short and intermediate PR+ patients, HbA_{1C}, $IDAA_{1C}$ and $GTAA_{1C}$ scores at +6 months were significantly lower (respectively p=0.02, p=0.02 and p=0.003) in long PR+ patients compared with PR- control group. Long PR+ patients also exhibited a notably higher percentage of normoglycemia (55.4 ± 12.2 % vs 43.8 ± 10.8 %, p<0.001), less time in hyperglycemia (34.5% ± 12.8 vs 42.0% ± 12.6, [p=0.03]) and hypoglycemia (10.4% vs 14.2%, [p=0.03]) compared with PR- children. Furthermore, PR+ patients showed lower mean blood glucose values (158.3 ± 27.3 mg/dL vs 172.5 ± 27.7 mg/dL, [p=0.03]) and glycemic variability (83.1 ± 19.6 mg/dL vs 95.8 ± 19.3 mg/dL, [p=0.01]) compared to the control group. No other differences were observed between both groups for other clinical or glycemic control parameters, including the occurrence of severe hypoglycemia events and the number of daily glycemic tests. There were no significant differences in the metabolic follow-up data at 12 months between long PR+ and PR- patients. Comparing both CGM and SMBG values demonstrate similar results for glucose homeostasis parameters (all p-values > 0.05, data not shown).

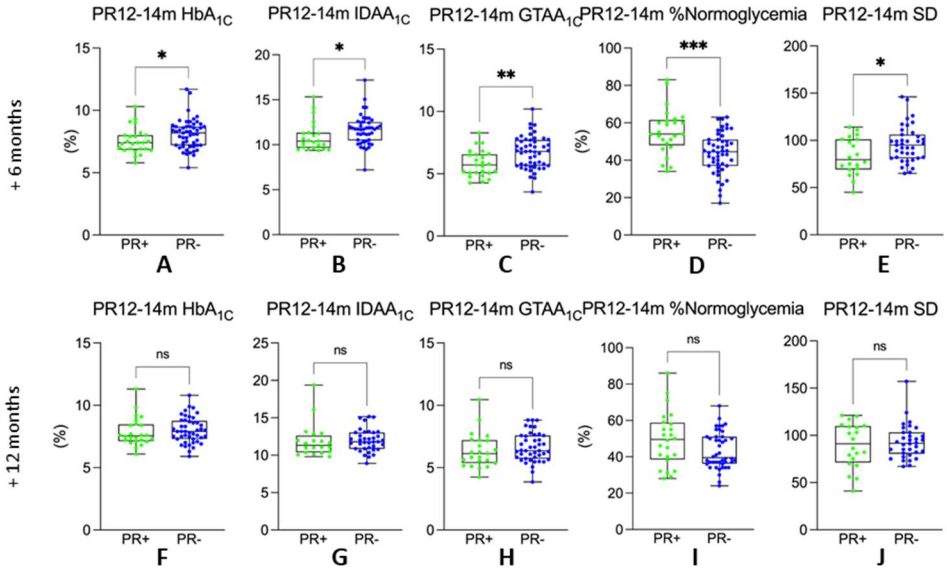

Figure 4: Assessment of the influence of long PR (>12-≤14 months) at 6- and 12-months post-remission. Comparison of the long PR^+ cohort to the control PR^- cohort, matched at the same post-diagnosis time for different parameters. Comparison of long PR^+ cohort at 6 months post-PR *vs* PR^- cohort at 18 months (A): for HbA_{1C}, (B): for $IDAA_{1C}$, (C): for $GTAA_{1C}$, (D): for TIR, (E): for glycemic variability. Comparison of intermediate PR^+ cohort at 12 months post-PR *vs* PR^- cohort at 24 months (F): for HbA_{1C}, (G): for $IDAA_{1C}$, (H): for $GTAA_{1C}$, (I): for TIR, (J): for glycemic variability. Box plots display the median, 25th and 75th percentiles, and range the different parameters between PR^+ group (green points) and PR^- group (blue points). Levels of significance are represented as follows: ns ($p>0.05$), * ($p<0.05$), ** ($p<0.01$), *** ($p<0.001$). PR: partial remission. HbA_{1C}: hemoglobin A1c. $IDAA_{1C}$: insulin dose-adjusted HbA_{1C}. $GTAA_{1C}$: glycemic target-adjusted HbA_{1C}. TIR: time in range. SD: standard deviation.

PR duration also influences diabetes metabolic control

The data of the different PR+ subgroups were cross-sectionally compared with each other at the same time post-diagnosis of diabetes. Children in long PR+ subgroup showed better glycemic control when compared to the short PR+ subgroup at 18 months post-diagnosis (+12 months from the end of PR for short PR+ children and +6 months for those with long PR) (**Fig. 5**). They

exhibited lower $GTAA_{1C}$ scores (p=0.02), better TIR (p=0.003), less time in hypoglycemia (10.45 ± 6.7 % vs 16.1 ± 11.1 %, [p=0.03]) and less glycemic variability (83.1 ± 31.8 mg/dL vs 98.84 ± 28.1 mg/dL, [p=0.03]) than short PR+ patients. No significant difference was observed for the other studied parameters. Finally, there was no significant difference when comparing short and intermediate PR+ subgroups.

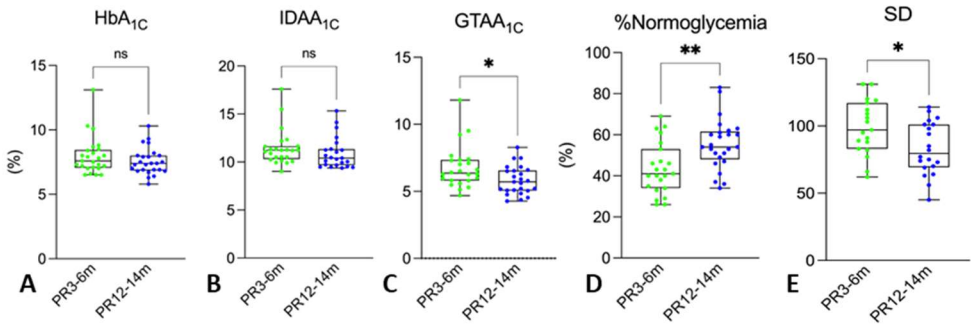

Figure 5: Comparison of long PR cohort (>12-≤14 months) at 6 months post-remission to short PR cohort (>3-≤6 months) at 12 months post-remission. Comparison (A): for HbA_{1C}, (B): for $IDAA_{1C}$, (C): for $GTAA_{1C}$, (D): for TIR, (E): for glycemic variability. Box plots display the median, 25th and 75th percentiles, and range the different parameters between PR^+ group (green points) and PR^- group (blue points). Levels of significance are represented as follows: ns (p>0.05), * (p<0.05), ** (p<0.01), *** (p<0.001). PR: partial remission. HbA_{1C}: hemoglobin A1c. $IDAA_{1C}$: insulin dose-adjusted HbA_{1C}. $GTAA_{1C}$: glycemic target-adjusted HbA_{1C}. TIR: time in range. SD: standard deviation.

3.8.5 Discussion

PR is a state of low glycemic variability, daily insulin needs and HbA_{1C} levels. Recent studies in young adults suggested that patients entering PR after diabetes onset were less at risk of vascular complications (25). Currently, little is known about the influence of PR and its duration on short-term glucose homeostasis outcomes, especially in children.

We first characterized the cohort according to remission status (i.e., PR+ and PR-). Next, we subdivided PR+ groups regarding the duration of the PR (i.e., short, intermediate and long) and evaluated whether the latter influenced glucose homeostasis at +6 and +12 months after the end of the PR. Globally, we found that patients experiencing PR had improved glycemic control at +6 months when compared to non-remitters, independently of the PR duration subgroups. Yet these results were not significant at +12 months. Finally, comparison between PR+ subgroups showed that experiencing a long PR allowed better glycemic control at +6 months compared to short PR.

Hallmarks of PR are a combination of low HbA_{1C}, low insulin daily doses and low glucose variability. In this context, both $IDAA_{1C}$ and $GTAA_{1C}$ scores highlight the positive influence of PR on diabetes control shortly after the end of PR (i.e., 6 months), each in a different way. An $IDAA_{1C}$ score ≤ 9 strongly correlates with a stimulated C-peptide level ≥ 300 pmol/L (7) reflecting residual β-cell secretion that characterizes PR (28). This score depends on HbA_{1C} and total daily insulin dose that reflects metabolic control in a broad sense. As previously suggested by our team (12), the $GTAA_{1C}$ score allows an evaluation of PR independently of insulin requirements and based on an objective measure in addition to HbA_{1C}: time in normoglycemia. $GTAA_{1C}$ provides a better reflection of glucose homeostasis and eventually a more clinically meaningful aspect of PR. Interestingly, $IDAA_{1C}$ and $GTAA_{1C}$ scores remained significantly lower in all three PR+ subgroups 6 months after the end of their PR period when compared to PR- patients.

Furthermore, the duration of PR influences the residual effect on glucose homeostasis: the longer the PR, the better the post-PR glycemic control at +6 months. Indeed, of all PR+ subgroups, long PR patients showed the best glucose homeostasis at 6 months after the end of the PR period with significantly lower $GTAA_{1C}$ score, better TIR and less glycemic variability, than in the short PR+ subgroup. Our results additionally support the importance of prolonging PR period, as long PR improved glucose homeostasis and duration of the residual effects when compared to short PR.

Current mechanisms underlying the residual effect of PR on short-term glucose homeostasis remain poorly understood. Partial remission is characterized by decreased glucotoxicity (i.e., decreased glucose variability and increased euglycemia (29)) decreased lipotoxicity (e.g., decreased LDL levels (30)) and increased immunotolerance (e.g., increased regulatory cells, upregulation of PDL-1 on lymphocytes (29,31,32)) jointly concurring to a reduction of β-cell destruction. These phenomena might together lead to improved residual secretion and increased insulin sensitivity that are known to have a long-term beneficial impact on micro- and macro-vascular diabetes complications (25) and are suspected to play a role in metabolic memory (33,34). We may assume that the combination of these mechanisms might also influence short-term glycemic control and influence the heterogeneity of PR duration. Taken together, these might partially explain the positive residual effect of PR on short-term metabolic balance that demonstrated to be proportional to its duration.

Another hypothesis behind this remanent phenomenon might be that it could be influenced by behavioral components (e.g., healthy diet and/or regular physical activity fostering residual insulin secretion). Yet this aspect of remanent stability of glucose homeostasis is difficult to demonstrate in our cohort, given the monocentric retrospective design of our study (all of our patients count carbohydrates and were given the same dietary education). Even though our study did not evaluate these aspects, it was previously shown that patients with type 1 diabetes who engage in regular physical activity have a higher incidence of PR (i.e., 44% vs 13%) and significantly higher prevalence of residual C-peptide 2 years after T1D onset (35). This would require further investigations in longitudinal follow-up studies of patients with new-onset type 1 diabetes (as in our DIATAG study protocol (36)).

Our initial hypothesis based on a clinical intuition was that a longer PR might be associated with a higher risk of glycemic imbalance shortly after PR ends as remitters would less be keen to strictly monitor their diabetes (and could adopt unhealthy habits during PR). Conversely, our results demonstrated that PR was significantly associated with improved glycemic control at 6 months. In addition, no differences were observed in the number of glycemic tests between remitters and non-remitters patients at 6 and 12 months post-PR (independently of the PR+ duration subgroup). This observation could support that the management of T1DM during the remission period (i.e., stability of glycemic control) did not modify the habits of glucose self-monitoring after PR ended.

Finally, it is also important to emphasize that there was no difference among PR+ subgroups regarding the occurrence of severe hypoglycemia when compared to children without remission (and no difference when comparing the three PR+ subgroups with each other). This supports recent results from our group which found no significant differences in the daily rate of grade II hypoglycemia (i.e., <54 mg/dL) between PR+ and PR- patients (Polle et al., Diabetes Care 2022, Accepted).

Our study demonstrates several strengths. To the best of our knowledge, our cohort is the largest pediatric population to study for the first time the influence of PR on short-term glycemic control. Implementing a long-term evaluation of the effects of PR duration in longitudinal cohorts of patients with T1DM would be required to confirm our findings. This could be the subject of a study in the context of a national register of children with diabetes in Belgian centers.

Our study also exhibits several limitations, the main one being the single-center retrospective design of our data collection. Although we focused on objective biological parameters (such as HbA_{1C} and the resulting $IDAA_{1C}$ score), glycemic follow-up data (even those collected from CGM methods) still partly depend on the regularity of individual daily monitoring. Moreover, as most patients were diagnosed with type 1 diabetes between 1997 and 2014 (implementation of CGM in Belgium), blood glucose data were not collected in a standardized way in all patients because we included patients with both glucometers and CGM. For this reason, we separately analyzed data collected from CGM and SMBG and obtained similar results in glucose

homeostasis parameters (all p-values > 0.05, data not shown). Also, the determination of PR duration is subject to longitudinal quarterly follow-up of patients, potentially impacting the accuracy of defining the exact end of PR period.

In conclusion, our study confirms the previously observed frequency of PR occurrence in European pediatric patients with type 1 diabetes (>60%) and the lowest incidence of PR in young-onset children (<5 years). Our results also emphasize the positive impact of this PR period on short-term metabolic control (better HbA_{1C} levels, $IDAA_{1C}$ and $GTAA_{1C}$ scores, TIR and less glycemic variability) without increasing the number of hypoglycemia. This favorable effect seems to last at least 6 months after remission, but a significant influence at 12 months post-PR was not observed. The duration of PR is nevertheless associated with a more pronounced residual effect: the longer the PR, the better the post-PR glycemic control (at 6 months). This supports that prevention protocols that aim at prolonging PR may also improve short-term metabolic control, even after PR ends.

Acknowledgements

The authors declare no conflicts of interest.

3.8.6 References

1. Van Belle TL, Coppieters KT, Von Herrath MG. Type 1 diabetes: Etiology, immunology, and therapeutic strategies. Physiol Rev. 2011;91(1):79–118.

2. Donath MY, Hess C, Palmer E. What is the role of autoimmunity in type 1 diabetes? A clinical perspective. Diabetologia. 2014;57(4):653–5.

3. Mayer-Davis EJ, Kahkoska AR, Jefferies C, Dabelea D, Balde N, Gong CX, et al. ISPAD Clinical Practice Consensus Guidelines 2018: Definition, epidemiology, and classification of diabetes in children and adolescents. Pediatr Diabetes. 2018;19:7–19.

4. Abdul-Rasoul M, Habib H, Al-Khouly M. "The honeymoon phase" in children with type 1 diabetes mellitus: Frequency, duration, and influential factors. Pediatr Diabetes. 2006;7(2):101–7.

5. Couper JJ, Haller MJ, Greenbaum CJ, Ziegler AG, Wherrett DK, Knip M, et al. ISPAD Clinical Practice Consensus Guidelines 2018: Stages of type 1 diabetes in children and adolescents. Pediatr Diabetes. 2018;19:20–7.

6. Lundberg RL, Marino KR, Jasrotia A, Maranda LS, Barton BA, Alonso LC, et al. Partial clinical remission in type 1 diabetes: a comparison of the accuracy of total daily dose of insulin of <0.3 units/kg/day to the gold standard insulin-dose adjusted hemoglobin A1c of ≤9 for the detection of partial clinical remission. J Pediatr Endocrinol Metab. 2017;30(8):823–30.

7. Mortensen HB, Hougaard P, Swift P, Hansen L, Holl RW, Hoey H, et al. New definition for the partial remission period in children and adolescents with type 1 diabetes. Diabetes Care. 2009;32(8):1384–90.

8. Max Andersen MLC, Hougaard P, Pörksen S, Nielsen LB, Fredheim S, Svensson J, et al. Partial Remission Definition: Validation based on the insulin dose-adjusted HbA1c (IDAA1C) in 129 Danish Children

with New-Onset Type 1 Diabetes. Pediatr Diabetes. 2014;15(7):469–76.

9. Nagl K, Hermann JM, Plamper M, Schröder C, Dost A, Kordonouri O, et al. Factors contributing to partial remission in type 1 diabetes: analysis based on the insulin dose-adjusted HbA1c in 3657 children and adolescents from Germany and Austria. Pediatr Diabetes. 2017;18(6):428–34.

10. Pecheur A, Barrea T, Vandooren V, Beauloye V, Robert A, Lysy PA. Characteristics and Determinants of Partial Remission in Children with Type 1 Diabetes Using the Insulin-Dose-Adjusted A1C Definition. J Diabetes Res. 2014;2014:1–7.

11. Chiavaroli V, Derraik JGB, Jalaludin MY, Albert BB, Ramkumar S, Cutfield WS, et al. Partial remission in type 1 diabetes and associated factors: Analysis based on the insulin dose-adjusted hemoglobin A1c in children and adolescents from a regional diabetes center, Auckland, New Zealand. Pediatr Diabetes. 2019;20(7):892–900.

12. Nielens N, Pollé O, Robert A, Lysy PA. Integration of Routine Parameters of Glycemic Variability in a Simple Screening Method for Partial Remission in Children with Type 1 Diabetes. J Diabetes Res. 2018;2018.

13. Unger RH, Grundy S. Hyperglycaemia as an inducer as well as a consequence of impaired islet cell function and insulin resistance: implications for the management of diabetes. Diabetologia. 1985 Mar;28(3):119–21.

14. Ozen G, Zanfardino A, Confetto S, Piscopo A, Casaburo F, Tinto N, et al. The Association of Autoimmune Diseases with Type 1 Diabetes Mellitus in Children Depends Also by the Length of Partial Clinical Remission Phase (Honeymoon). Int J Endocrinol. 2020;2020.

15. Bowden SA, Duck MM, Hoffman RP. Young children (< 5 yr) and adolescents (> 12 yr) with type 1 diabetes mellitus have low rate of partial remission: Diabetic ketoacidosis is an important risk factor. Pediatr Diabetes. 2008;9(3 PART 1):197–201.

16. Zhong T, Tang R, Gong S, Li J, Li X, Zhou Z. The remission phase in

type 1 diabetes: Changing epidemiology, definitions, and emerging immuno-metabolic mechanisms. Diabetes Metab Res Rev. 2020;36(2):0–2.

17. Dost A, Herbst A, Kintzel K, Haberland H, Roth CL, Gortner L, et al. Shorter remission period in young versus older children with diabetes mellitus type 1. Exp Clin Endocrinol Diabetes. 2007;115(1):33–7.

18. Kara Ö, Esen İ, Tepe D. Factors influencing frequency and duration of remission in children and adolescents newly diagnosed with type 1 diabetes. Med Sci Monit. 2018;24:5996–6001.

19. Schölin A, Törn C, Nyström L, Berne C, Arnqvist H, Blohmé G, et al. Normal weight promotes remission and low number of islet antibodies prolong the duration of remission in Type 1 diabetes. Diabet Med. 2004;21(5):447–55.

20. Marino KR, Lundberg RL, Jasrotia A, Maranda LS, Thompson MJ, Barton BA, et al. A predictive model for lack of partial clinical remission in new-onset pediatric type 1 diabetes. PLoS One. 2017;12(5):e0176860.

21. Schloot NC, Hanifi-Moghaddam P, Aabenhus-Andersen N, Alizadeh BZ, Saha MT, Knip M, et al. Association of immune mediators at diagnosis of Type 1 diabetes with later clinical remission. Diabet Med. 2007 May;24(5):512–20.

22. Wong TWC, Wong MYS, But WMB. Features of partial remission in children with type 1 diabetes using the insulin dose-adjusted A1c definition and risk factors associated with nonremission. Ann Pediatr Endocrinol Metab. 2021 Jun 30;26(2):118–25.

23. Nathan DM. Realising the long-term promise of insulin therapy : the DCCT / EDIC study Diabetes Control and Complications Trial. 2021;1049–58.

24. Nathan DM. The diabetes control and complications trial/epidemiology of diabetes interventions and complications study at 30 years: Overview. Diabetes Care. 2014;37(1):9–16.

25. Niedzwiecki P, Pilacinski S, Uruska A, Adamska A, Naskret D, Zozulinska-Ziolkiewicz D. Influence of remission and its duration on development of early microvascular complications in young adults with type 1 diabetes. J Diabetes Complications. 2015 Nov;29(8):1105–11.

26. Roelants M, Hauspie R, Hoppenbrouwers K. References for growth and pubertal development from birth to 21 years in Flanders, Belgium. Ann Hum Biol. 2009;36(6):680–94.

27. Abraham MB, Jones TW, Naranjo D, Karges B, Oduwole A, Tauschmann M, et al. ISPAD Clinical Practice Consensus Guidelines 2018: Assessment and management of hypoglycemia in children and adolescents with diabetes. Pediatr Diabetes. 2018 Oct 1;19:178–92.

28. The Diabetes Control and Complications Trial Research Group. Effect of Intensive Therapy on Residual β-Cell Function in Patients with Type 1 Diabetes in the Diabetes Control and Complications Trial. Ann Intern Med. 1998 Apr 1;128(7):517.

29. Fonolleda M, Murillo M, Vázquez F, Bel J, Vives-Pi M. Remission Phase in Paediatric Type 1 Diabetes: New Understanding and Emerging Biomarkers. Horm Res Paediatr. 2017;88(5):307–15.

30. Nwosu BU, Zhang B, Ayyoub SS, Choi S, Villalobos-Ortiz TR, Alonso LC, et al. Children with type 1 diabetes who experienced a honeymoon phase had significantly lower LDL cholesterol 5 years after diagnosis. Pietropaolo M, editor. PLoS One. 2018 May 16;13(5):e0196912.

31. Li X, Zhong T, Tang R, Wu C, Xie Y, Liu F, et al. PD-1 and PD-L1 Expression in Peripheral CD4/CD8+ T Cells Is Restored in the Partial Remission Phase in Type 1 Diabetes. J Clin Endocrinol Metab. 2020 Jun 1;105(6):1947–56.

32. Fitas AL, Martins C, Borrego LM, Lopes L, Jörns A, Lenzen S, et al. Immune cell and cytokine patterns in children with type 1 diabetes mellitus undergoing a remission phase: A longitudinal study. Pediatr Diabetes. 2018 Aug 1;19(5):963–71.

33. Testa R, Bonfigli AR, Prattichizzo F, La Sala L, De Nigris V, Ceriello A.

The “Metabolic Memory” Theory and the Early Treatment of Hyperglycemia in Prevention of Diabetic Complications. Nutrients. 2017 Apr 28;9(5).

34. Sherry NA, Tsai EB, Herold KC. Natural History of β-Cell Function in Type 1 Diabetes. Diabetes. 2005 Dec 1;54(suppl_2):S32–9.

35. Jamiołkowska-Sztabkowska M, Głowińska-Olszewska B, Łuczyński W, Konstantynowicz J, Bossowski A. Regular physical activity as a physiological factor contributing to extend partial remission time in children with new onset diabetes mellitus—Two years observation. Pediatr Diabetes. 2020;21(5):800–7.

36. Pollé OG, Delfosse A, Martin M, Louis J, Gies I, den Brinker M, et al. Glycemic Variability Patterns Strongly Correlate With Partial Remission Status in Children With Newly Diagnosed Type 1 Diabetes. Diabetes Care. 2022;45(October):2360–8.

Table 1. Characteristics of the patients at diagnosis according to partial remission (PR) status at 3 months post-diagnosis.

	Total (n=189)	PR^+ at 3 months (n=132)	PR^- at 3 months (n=57)	*p-values*
Gender – n (%)	**95 (50.26)**	**68 (71.58)**	**27 (28.42)**	**0.64** [a]
Age (years)				
Mean ± SD (years) °	**9.01 ± 3.68**	**9.61 ± 3.43**	**7.64 ±3.92**	**0.001** [b]
<5 – n (%)	**32 (16.93°)**	**14 (43.75)**	**18 (56.25)**	**NA**
5-12 – n (%)	**116 (61.38°)**	**85 (73.28)**	**31 (26.72)**	**NA**
>12 – n (%)	**41 (21.69°)**	**33 (80.49)**	**8 (19.51)**	**NA**
BMI (Z-score) °	**0.3 ± 1.1**	**0.2 ± 1.1**	**0.3 ± 1.3**	**0.66** [b]

Legend: [a] Chi-square test. [b] Student t-test. Abbreviations: PR = partial remission. BMI = Body Mass Index. SD = standard deviation

3.8.7 Supplemental material

	Short PR^+ (≥3-≤6 months)	Intermediate PR^+ (>6-≤12 months)	Long PR^+ (>12-≤14 months)
PR^- 12 months	+6 months post PR **(Fig. 2)**		
PR^- 15 months		+6 months post PR **(Fig. 3)**	
PR^- 18 months	+12 months post PR **(Fig. 2)**		+6 months post PR **(Fig. 4)**
PR^- 21 months		+12 months post PR **(Fig. 3)**	
PR^- 24 months			+12 months post PR **(Fig. 4)**

Supplemental Figure S1 : Schematic representation of partial remission period comparisons. PR^+ patients were divided in three subgroups according to PR duration (short, intermediate and long PR) and compared at +6 and +12 months after the end of their PR period, to PR^- patients at the same time points after diagnosis. PR^+ patients with short PR were also compared with intermediate and long PR^+ patients (not represented on the table). PR: Partial Remission.

4 General Discussion

Preservation of residual β-cell function after the T1D onset is associated with improved glycemic outcomes (e.g., HbA_{1C}, reduced hypoglycemic excursions) and reduced microvascular complications (268–273). Current prevention strategies aiming to reduce β-cell loss after T1D onset yielded some encouraging results (69,227) yet extensive gaps remain in the understanding of disease physiopathology. Indeed, these studies also shed light on poorly characterized areas of T1D currently hampering the progress towards disease-modifying therapies and include alterations of the exocrine pancreas, clinical heterogeneity among patients (e.g., PR), and the lack of both clinically reliable and predictive biomarkers of β-cell function.

Our results supported the importance of extending PR after T1D onset as both its occurrence and duration improved short and mid-term glucose homeostasis and health-related quality of life. Focusing on a better characterization of T1D, CGM, and pancreatic MRI provided additional clues to the clinical heterogeneity observed in patients during the first year following the clinical onset (e.g., glucotypes, age-driven differences, and longitudinal evolution of pancreas morphology). Also, our data questioned residual stimulated C-peptide as a clinically reliable marker of β-cell function and proposed new minimal-invasive alternatives for prevention trials (i.e., CGM metrics [early morning values in specified glycemic ranges and PHH] or MRI indices). Finally, multiparametric MRI assessment of pancreas and plasmatic proteomes at T1D diagnosis moderately predicted the evolution of glucose homeostasis and took the first steps into patient stratification.

PR is a key period to initiate therapeutic interventions as metabolic and immune processes surrounding the period facilitate the resurgence of residual β-cell function (139,202,274). Despite being transient in time, our results supported that patients experiencing the longest remission period achieved better short-term glycemic balance when PR ends (i.e., $IDAA_{1C}$, $GTAA_{1C}$, TIR, and HbA_{1C} at 6 months post-PR). Our work corroborated previous observations that showed better long-term glycemic control (e.g., decrease of HbA_{1C}, TDD), a higher β-cell residual secretion, and a reduction of microvascular complications in patients that underwent PR (173,191,193). Hypotheses beyond the residual effect of PR remain debated and may include physiological aspects (e.g., metabolic memory) (195,196), behavioral components (e.g., regular sport, healthy diet) (200), and the presence of specific patient phenotypes.

Multilevel approach is key to better understand the physiopathology of T1D and identify clinically meaningful markers to individualize prevention therapies (138,169,244). Following this rationale, the DIATAG trial integrated both fundamental and clinical data to perform cross-sectional comparisons among pediatric patients with new-onset T1D. A nearly similar approach was performed by the European “Innovative Approach Towards Understanding and Arresting Type 1 Diabetes” (INNODIA) infrastructure which aimed at characterizing the natural history of patients following the onset of T1D and identifying new biomarkers of β-cell mass evolution (275). As highlighted by the INNODIA consortium workflow, the quality of multilevel data integration relies both on the utilization of standard operating procedures (SOP) across participating centers and the

centralization of most analyses (275). As an example, set-up tests were performed in DIATAG to investigate the effect of sample retrieval on the abundance of different lymphocyte subpopulations (Treg, Th1, Th2, Th17, Tfr and Tfh) (Julie Lemmer master thesis, *data not shown*). Corroborating previous findings (276), most lymphocyte subpopulations remained stable when whole blood was kept at room temperature during the retrieval and PBMCs were isolated within 24 hours of sampling (Julie Lemmer master thesis, *data not shown*). In our study, isolation of PBMCs was thus centralized in CUSL for most participating centers and performed on the same day as the venous puncture.

Prevention trials also need clinically meaningful readouts. In most residual β-cell mass tertiary prevention studies, primary outcome is defined by residual stimulated C-peptide levels at least 1-year postdiagnosis (277). However, while some trials showed a significant effect on C-peptide levels, changes in routine markers of glucose homeostasis were less likely observed, with few patients showing lower HbA_{1C} and even fewer showing lower TDD (277). Furthermore, most of these tertiary prevention trials did not evaluate the modifications of key glucose homeostasis parameters such as glycemic variability and hypoglycemia (277,278). Our team and others showed that stimulated C-peptide incompletely reflected β-cell function and needed further standardization (168,275). Similarly to previous studies, discrepancies were found between residual stimulated C-peptide values and both the glucose homeostasis (e.g., HbA_{1C}, $IDAA_{1C}$, TIR) (220,221) and phenotype of the patient (e.g., remission status) (168). Part of these inconsistencies may rely, at least partially, on individual factors such as

insulin sensitivity and β-cell glucose responsiveness (220,272,279). These results together challenge residual C-peptide as a forefront marker of β-cell function and support the need for new biomarkers to stratify patients (e.g., glucose homeostasis).

- **Preserving residual β-cell function after T1D onset is important to improve both short and long-term glucose homeostasis.**
- **Characterization of heterogeneous diseases such as T1D must rely on multilevel approaches and cross-sectional integration of the data.**
- **Evaluation of treatment response in prevention trials must additionally rely on clinically meaningful markers of β-cell function other than C-peptide.**

Promoting this idea, we showed that early morning CGM values distinguished remitters from non-remitters and witnessed residual β-cell function after clinical onset (i.e., values in 63-70mg/dL range). These data were supported by previous punctual observations that showed frequent $TBR_{<70}$ in secretors (29), minimal increase in $TBR_{<70}$ in remitters (33), and a high proportion (>50%) of $TBR_{<70}$ in the 65 to 70 mg/dL range during PR (33). Extending the current panel of commonly used CGM metrics, we described PHH parameters (e.g., PHH hyper ratio and PHH frequency) (258) as additional markers of glucose homeostasis (including glucose variability) and PR. Interestingly, values of PHH parameters showed an exponential increase

from CV of 36% and independently validated the diabetes stability threshold (280). Finally, supporting PHH as a marker of microvascular complications, similar glycemic patterns were associated with both increased circulating markers of inflammation and oxidative stress, and decreased endothelial function in patients with T1D (281). These findings together reinforce CGM as an additional minimal-invasive tool to evaluate glycemic balance, residual β-cell function, and potentially glucose-related oxidative stress.

Our CGM and plasma proteomics findings added knowledge to the current $IDAA_{1C}$-based definition of PR. On the one hand, most CGM metrics showed an overlap in remission status for $IDAA_{1C}$ values in the 8 and 10 range (168). On the other hand, differential analysis of plasmatic proteomes yielded significant results only when considering $IDAA_{1C}$ as a continuous scale. These data support the limitations of PR definition when based on a threshold (i.e., $IDAA_{1C}$ score of 9) as it fails to integrate the intensity of PR and may lead to the misclassification of patients with intermediate scores. Indeed, individual factors such as insulin sensitivity and patient compliance to the treatment (influence of TDD reporting) may influence the $IDAA_{1C}$ score and limit its reliability (e.g., underestimation of PR in pubertal girls and before the first 3 months after T1D onset) (175,177–180).

Integration of clinical parameters and CGM metrics supported PR as a continuous phenomenon. Four clinically-relevant glucotypes were identified during the first year following T1D diagnosis (168) and subsequently validated by PHH parameters (see **4.1** and **4.2**). These distinct glycemic patterns supported the intuitive feeling that the

progressive decline of β-cell function led to dysglycemia first emerging in the context of carbohydrates intake (i.e., daytime) before extending to carbohydrates-free periods (i.e., nighttime). Indeed, the onset of hyperglycemic excursions and increased glycemic variability during the first year of T1D may first result from the progressive decrease of β-cell function. In patients from glucotype 2, the persistence of intermediate β-cell function prevented grade II hyperglycemia (especially during nighttime) and maintained a CV below 36% during the whole day. When β-cell function further declines, additional factors such as diabetes self-management (e.g., compliance burden) and hypoglycemia overtreatment also participate to the glucose disbalance. Supporting this idea, our most dysregulated glucotypes (i.e., 3 and 4) were characterized by specific PHH parameters (e.g., PHH_{AUC} and PHH/Hypoglycemia frequency ratio) and previously described in children with long-term T1D (255).

- **Early morning CGM metrics values and PHH parameters are clinically meaningful and minimal-invasive markers of PR and β-cell function.**
- **$IDAA_{1C}$ score shows limitations especially for values in 8-10 range.**
- **PR should be considered as continuum rather than a binary condition.**
- **Glucotypes witness the progressive appearance of glycemic dysregulations and were independently validated by PHH parameters.**

Going further into the characterization of pediatric T1D patchiness, our results provided new clues in the characterization of previously described age-related T1D endotypes (see **4.1** and **4.3**) and supported the existence of radio-clinic differences between children with prepubertal and pubertal onset. Compelling evidence suggested a more aggressive disease in patients with young T1D onset. Indeed, patients aged below 7 years displayed more aggressive histological characteristics (161,162), more severe clinical phenotype (137,159,161,165), and carried higher-risk genotypes (163). Our findings strengthened this concept as children with prepubertal T1D onset (representing 50% of our MRI cohort) showed increased pancreas atrophy as compared to pubertal onset. Age-related

disparities were also observed in the evolution of glucose homeostasis in both our DIATAG and DIABHONEY cohorts. Patients with a younger age at T1D onset experienced significantly fewer PR and were mostly distributed in most dysregulated glucotypes (i.e., 3 and 4) at three months postdiagnosis. Reduced incidence of PR was similarly observed in children with young onset, especially below 5 years of age (177,262,282).

Our results provided additional evidence of whole pancreatic disease in T1D (see **4.3**). Corroborating previous findings (283–285), patients with new-onset T1D demonstrated increased pancreas atrophy as compared to matched healthy controls (approximately 50%). Noteworthy, this atrophy was homogeneous across pancreatic subregions and supported previous *in* vivo and histological findings that showed global inflammation and immune infiltration in most new-onset T1D pancreas (133,157,286,287). In non-T1D patients, evidence suggested a heterogeneous distribution of endocrine function in the pancreas tissue. Indeed, histological and clinical observations such as increased β-cell area, higher incidence of diabetes following distal pancreatotomy, and similar glucose homeostasis following pancreas head resection supported the presence of a higher proportion of endocrine cells in the pancreatic tail of healthy patients (288–291). Our findings provided new insights into T1D population with moderate topographic correlations being observed between PV_{TAIL} with $CPEP_{EST}$ and PV_{HEAD} with trypsinogen. Nonetheless, whole PV indices were highly heterogeneous among patients and did not correlate with diabetes severity at diagnosis or markers of glucose homeostasis during the 1rst year of T1D. These observations altogether suggest that atrophy of exocrine pancreas may result from both

β-cell decline and additional heterogeneous phenomena that are captured by current markers of T1D follow-up (106).

Finally, prediction of β-cell function evolution is important for the stratification of patients at T1D onset (see **4.3** and **4.4**). On the one hand, the combination of topographic MRI indices and clinical characteristics of the patient allowed the identification of pancreatic functions prediction models during the first year postdiagnosis. These observations support the role of pancreatic multiparametric MRI assessment and topographic analysis in the field of T1D (292). While disparities exist in the delineation of pancreatic subregions and MRI acquisition methods, the MAP-T1D initiative (256) recently set the groundwork for the standardization of MRI measurements across studies (292,293). On the other hand, the abundance of 26 plasmatic proteins moderately correlated to $IDAA_{1C}$ score at +3 months. Around clinical onset, patients with T1D experience a global metabolic disturbance characterized by hyperglycemia, hyperlipemia, global inflammation, and eventually acidosis that may be associated with alterations of the coagulation, decrease in muscle mass, increased cellular stress, and β-cell dysfunction (16,294). Importantly, some of our protein candidates (n=11) were associated with at least one of these T1D-related phenomena though being ubiquitously expressed. Variation in their plasmatic levels may thus witness a global condition affecting the entire metabolism rather than an islet-specific protein release (i.e., 2% of pancreatic mass).

- **Children with prepubertal T1D onset have a more aggressive disease phenotype (endocrine and exocrine).**
- **T1D implicates both endocrine and exocrine pancreatic compartments.**
- **Topographic correlations between pancreatic functions and PV indices support the heterogeneous distribution of pancreatic β cells.**
- **Plasmatic proteome and MRI markers are clinically relevant for disease stratification**

Limitations

DIATAG is a multicentric study including seven centers in Belgium located in a 100km perimeter around CUSL (i.e., central laboratory). Therefore, most pre-analytical treatments of blood and urine samples were performed on-site. Despite the application of similar pretreatment SOP across all participating centers (275), site-specific laboratory materials (e.g., centrifugation) and staff may lead to batch effects. Data quality check (i.e., plasma proteomic study) (295) and utilization of linear mixed models prevented a part of these biases. However, as observed in the plasmatic miRNA study, influences of the latter persist (*data not included in this thesis*).

Another limitation of our studies is the partially incomplete character of our cohort. In the DIATAG trial, we observed few dropouts (i.e., 7/98) and patients missing follow-up visits due to the COVID-19 pandemia. Also,

DIATAG trial is still open concurring to incomplete follow-up data in most recent participants. Therefore, cross-sectional analyses of diabetes-related parameters (i.e., clinic, secretion, CGM including PHH patterns) did not include the four time points for all individuals (i.e., 70%). Nonetheless, the impact on the results was limited as both CGM studies aimed to depict glucotypes or specific glycemic patterns rather than evaluating the individual evolution of patients. Another cause of missing data was the incomplete adherence to the full GST testing due to side effects (study protocol offered the possibility of not undergoing the test). Impact of missing β-cell secretion data was nonetheless limited as $CPEP_{EST}$ allowed reliable secretion measurement for the great majority of the patients (>95%). Regarding the subsidiary MRI study, only a subset of patients underwent pancreatic imaging as MRI required a journey to CUSL (32%, n=31/98). Importantly, the MRI cohort showed similar characteristics to the whole DIATAG cohort and represents one of the biggest and youngest pediatric populations analyzed (110,284,296).

Glucose monitoring devices differed among patients included in both DIATAG and DIABHONEY trials. For CGM studies, the sensor manufacturer may influence the data though most of our DIATAG dataset (i.e., >90%) was obtained from Freestyle Libre®. For the retrospective DIABHONEY study, data included both SMBG and CGM data as first patients dated back to 1997. For this reason, we performed subanalysis according to glucose monitoring devices (i.e., CGM and SMBG) and obtained similar results for all glucose homeostasis parameters (all p-values > 0.05). Nonetheless, subgroup analyses reduce statistical power and may partially explain the loss PR

residual effect at +12 months in our results, though trends were observed. Other reasons underlying this observation may include the inhomogeneity of blood monitoring devices between groups and data collection. Evaluation of PR residual effect in patients of DIATAG cohort (when all patients reached 1-year post-PR) may provide an accurate validation step.

Our proteomic study also demonstrated limitations. The discovery phase was limited by the presence of an outlier ($IDAA_{1C}$ score = 14) which influenced linear regressions between protein abundance and $IDAA_{1C}$ score. Also, as first analysis was conducted on a low-abundance protein-enriched fraction of plasma, proteome of PM elutions may not reflect the raw plasma composition (i.e., aspecific loss of most abundant proteins). Nonetheless, a recent study showed that both eluted and bound fractions of PM showed mostly similar protein profiles (297). In line with this statement, our first validation phase using raw plasma targeted proteomics in the same 16 patients confirmed some of our candidates with (n=11) and without the presence of the outlier (n=5). Nonetheless, these results must undergo additional validations on a bigger and independent cohort (**see 6**).

Finally, the cost (i.e., >500 euros per patient) and availability of MRI may limit its translation into clinical practice for patients with T1D. Indeed, summarizing current cost-benefice balance of pancreatic MRI in T1D, we may support that cost is currently tipping the balance over if used in a clinical routine setting. Nonetheless, standardization of multiparametric MRI and development of automatic segmentation protocols may allow larger-scale studies (256) and participate in the understanding of T1D (293,298).

5 Further steps and perspectives

5.1 Bridging technologies to achieve a better characterization of T1D heterogeneity

In DIATAG trial, combination of demographic characteristics, clinical parameters of glucose homeostasis, residual β-cell secretion tests, CGM data, plasmatic proteomic, circulating miRNA, and MRI parameters improved the characterization of T1D patchiness.

First, the combination of CGM data, β-cell secretion, and routine clinical parameters allowed more accurate evaluation of glucose homeostasis. For example, the inclusion of both glycemic variability and hypoglycemia parameters differentiated patients with similar HbA_{1C} and TAR values, and intermediate $IDAA_{1C}$ scores into glucotypes 2 and 3 (168). These observations further supported the need for the medical team to integrate both clinical parameters (e.g., HbA_{1C}, TDD) and CGM metrics (including glycemic variability and hypo) when evaluating the glucose homeostasis of a patient. Moreover, CGM metrics allowed an accurate distinction of patients among the four glucotypes from 3 months postdiagnosis using 30-day data. These findings highlight that CGM, in combination with clinical parameters, may identify specific glycemic patterns shortly after T1D onset and help the stratification of patients. Finally, our team is currently exploring each patient's evolution across the glucotypes to better characterize the heterogeneity of glucose homeostasis dynamics during the first year of T1D.

Second, observations arising from our MRI study showed high inter-patient variability in pancreatic atrophy both at the onset and during the first

year of T1D. Mechanisms underlying pancreatic exocrinopathy remain unknown and do not correlate with markers of diabetes follow-up. Plasmatic miRNAs were recently described as potential circulating biomarkers in a number of disease including T1D (e.g., markers of β-cell stress and/or destruction) (275,299,300). These provided new clues in T1D physiopathology though relationships with the exocrine pancreas was never investigated. In this context,we are currently performing a cross-analysis between plasmatic miRNA expression (i.e., miRNA sequencing) and MRI indices in a subset of our patients (n=18) to identify potential mechanisms underlying pancreas atrophy.

Finally, our results extended the description of previously age-related endotypes to specific CGM patterns and pancreatic MRI characteristics. We hope that our findings will provide additional evidence for integrating the pubertal status as a key element in the (sub)analysis of residual β-cell prevention studies and the stratification of patients with new-onset T1D.

5.2 CGM may provide additional β-cell function markers for prevention studies

Evaluation of residual β-cell secretion using stimulation tests (e.g., MMTT or GST) is invasive and incompletely reflects the glucose homeostasis of the patient (see **1.4** and **4.1**). In our work, we propose specific CGM metrics as poorly invasive and clinically reliable alternatives to measure residual β-cell function (i.e., early morning values in 63-70 mg/dL range). Sustaining the translationality of our methodology, the calculation of these

markers relies on standardized, free and user-friendly software (e.g., Iglu and cgmanalysis packages). Bringing these results a step further, we think that the integration of CGM metrics as additional endpoints in residual β-cell function prevention trials (first as a secondary outcome) is essential to provide clinically relevant clues to evaluate treatment response. Also, we hope that our study and others (166,168,220,221,301,302) will rise awareness of medication agencies (e.g., European medicines agency and U.S. Food and drug agency) on the limitations of the current primary outcome (i.e., MMTT-derived residual β-cell secretion) and provide minimal-invasive alternatives. Implementation of CGM metrics in prevention trials may participate in the reduction of current burden of research protocols including multiple MMTT testing. Going further, we hope that it will help to improve patient inclusion and adherence to protocols, and foster the development of curative therapies for T1D.

5.3 Integration of PHH characteristics improves awareness and management of hypoglycemic excursions

Clinicians currently rely on ambulatory glucose profile (AGP) and HbA_{1C} values for the routine management of diabetes. AGP summarizes several CGM metrics (i.e., TBR, TIR, TAR, CV) and provides a good overview of the patient's glucose homeostasis. However, multiple causes may underly a similar dysglycemic state (e.g., hypoglycemia (303)) which may not be captured by current metrics. From that perspective, our team developed a freely available algorithm that allowed standardized calculations of the PHH parameters from all types of raw CGM data. The combination of PHH_{AUC}, PHH/Hypoglycemia frequency ratio, and hypoglycemia frequency yielded

the identification of three different groups with specific hypoglycemia profiles (see **4.2**). Among the latter, the majority of patients in Group 2 frequently overtreated their hypoglycemia when trying to reach a normoglycemic state, resulting in short and frequent PHH, and consequently high glycemic variability. In these patients, medical team should focus their therapeutic education on hypoglycemia management and aim to reduce PHH/Hypoglycemia frequency ratio below 0.25. Altogether, these data suggest that inclusion of PHH parameters into AGP will raise awareness of the causes underlying hypoglycemic excursions and provide an additional tool for physicians to foster patient-tailored interventions.

5.4 Prediction of PR may improve the stratification of patients at diagnosis

Focusing on plasma proteomic and pancreatic MRI at diagnosis, we identified markers that moderately predicted the $IDAA_{1C}$ score during the first year of T1D. On the one hand, early identification of remitters may allow the inclusion of patients having a high residual β-cell function in prevention trials. On the other hand, identification of non-remitters may allow the medical team to focus on high-risk patients that may benefit from early strict management of T1D (i.e., physical activity and tight glycemic control). Altogether, the stratification of patients at T1D diagnosis according to their propensity to enter PR may provide an additional decision tool to physicians for the early management of patients with new-onset T1D. Preservation of residual β-cell function after clinical onset is important as it both improves glucose homeostasis and partially reduces the burden of intensive insulin

therapy thereby enhancing the quality of life of both the patient and its parents.

6 Conclusion

Better characterization of T1D heterogeneity at clinical onset is key to achieving patient-tailored and global medical interventions. Currently, prevention trials lack precision in the stratification of patients and the monitoring of treatment responses. Identification of biomarkers that reliably reflect and/or predict the evolution of residual β-cell function is deeply needed.

In our work, we performed longitudinal and cross-sectional analyses of several markers including phenotypic characteristics of the patients, evaluation of both new and old markers of glucose homeostasis, measurement of the exocrine pancreatic function, and MRI of the pancreas during the first year following T1D onset. These findings added to the knowledge of previously described T1D endotypes (i.e., young *vs* old onset), challenged the binary definition of PR, and identified new clinically meaningful markers of β-cell function. Interestingly, most of these markers were evaluated using minimally invasive methods and may be easily implemented into the clinical routine. Going further into the stratification of patients at diagnosis, our studies identified short-term predictive markers of glucose homeostasis and PR. Finally, the retrospective analysis of new-onset pediatric T1D cohort provided additional clues supporting the residual effect of PR on short-term glucose homeostasis.

Overall, our observations underline the need for patient stratification around T1D diagnosis and inclusion of pubertal status in prevention trial subanalyses. Furthermore, our results challenged the residual C-peptide as

a primary endpoint of prevention studies and promoted CGM metrics as both new poorly invasive and clinically reliable alternatives.

7 Bibliography

1. Banting FG, Best CH, Collip JB, Campbell WR, Fletcher AA. Pancreatic Extracts in The Treatment of Diabetes Mellitus. Diabetes. 1956 Jan 1;5(1):69–71.
2. Nathan DM. Realising the long-term promise of insulin therapy : the DCCT / EDIC study Diabetes Control and Complications Trial. 2021;1049–58.
3. Diabetes N, Group D. Classification and Diagnosis of Diabetes Mellitus and Other Categories of Glucose Intolerance. Diabetes. 1979;28.
4. Gale EAM. The Discovery of Type 1 Diabetes. Diabetes. 2001 Feb 1;50(2):217–26.
5. Redondo MJ, Hagopian WA, Oram R, Steck AK, Vehik K, Weedon M, et al. The clinical consequences of heterogeneity within and between different diabetes types. Diabetologia. 2020;63(10):2040–8.
6. Gale EAM. Is type 2 diabetes a category error? Lancet. 2013;381(9881):1956–7.
7. Xu G, Liu B, Sun Y, Du Y, Snetselaar LG, Hu FB, et al. Prevalence of diagnosed type 1 and type 2 diabetes among US adults in 2016 and 2017: population based study. BMJ. 2018 Sep 4;362:k1497.
8. Drucker DJ. Transforming type 1 diabetes: the next wave of innovation. Diabetologia. 2021 May 6;64(5):1059–65.
9. Loriaux DL. Diabetes and The Ebers Papyrus. Endocrinologist. 2006 Mar;16(2):55–6.
10. Himsworth HP. DIABETES MELLITUS. ITS DIFFERENTIATION INTO INSULIN-SENSITIVE AND INSULIN-INSENSITIVE TYPES. Lancet. 1936 Jan 18;227(5864):127–30.
11. Nerup J, Platz P, Andersen OO, Christy M, Lyngsøe J, Poulsen J., et al. HL-A ANTIGENS AND DIABETES MELLITUS. Lancet. 1974 Oct;304(7885):864–6.
12. Bottazzo GF, Florin-Christensen A, Doniach D. Islet-Cell Antibodies in

Diabetes Mellitus With Autoimmune Polyendocrine Deficiencies. Lancet. 1974;304(7892):1279–83.

13. Eisenbarth GS. Type I Diabetes Mellitus. N Engl J Med. 1986 May 22;314(21):1360–8.

14. Insel RA, Dunne JL, Atkinson MA, Chiang JL, Dabelea D, Gottlieb PA, et al. Staging presymptomatic type 1 diabetes: A scientific statement of jdrf, the endocrine society, and the American diabetes association. Diabetes Care. 2015;38(10):1964–74.

15. Battaglia M, Ahmed S, Anderson MS, Atkinson MA, Becker D, Bingley PJ, et al. Introducing the endotype concept to address the challenge of disease heterogeneity in type 1 diabetes. Diabetes Care. 2020;43(1):5–12.

16. DiMeglio LA, Evans-Molina C, Oram RA. Type 1 diabetes. Lancet. 2018 Jun;391(10138):2449–62.

17. Cousin E, Duncan BB, Stein C, Ong KL, Vos T, Abbafati C, et al. Diabetes mortality and trends before 25 years of age: an analysis of the Global Burden of Disease Study 2019. Lancet Diabetes Endocrinol. 2022 Mar 1;10(3):177–92.

18. Patterson CC, Karuranga S, Salpea P, Saeedi P, Dahlquist G, Soltesz G, et al. Worldwide estimates of incidence, prevalence and mortality of type 1 diabetes in children and adolescents: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. Diabetes Res Clin Pract. 2019 Nov;157:107842.

19. Morgan E, Cardwell CR, Black CJ, McCance DR, Patterson CC. Excess mortality in Type 1 diabetes diagnosed in childhood and adolescence: a systematic review of population-based cohorts. Acta Diabetol. 2015;52(4):801–7.

20. Chiang JL, Maahs DM, Garvey KC, Hood KK, Laffel LM, Weinzimer SA, et al. Type 1 diabetes in children and adolescents: A position statement by the American Diabetes Association. Diabetes Care. 2018;41(9):2026–44.

21. Delamater AM, de Wit M, McDarby V, Malik JA, Hilliard ME, Northam E, et al. ISPAD Clinical Practice Consensus Guidelines 2018: Psychological care of children and adolescents with type 1 diabetes. Pediatr Diabetes. 2018;19:237–49.

22. Rewers MJ, Pillay K, de Beaufort C, Craig ME, Hanas R, Acerini CL, et al. Assessment and monitoring of glycemic control in children and adolescents

with diabetes. Pediatr Diabetes. 2014;15(SUPPL.20):102–14.

23. Saiyed M, Hasnani D, Alonso GT, Richmond E, Besançon S, Cotterill A, et al. Worldwide differences in childhood type 1 diabetes: The SWEET experience. Pediatr Diabetes. 2021 Mar 22;22(2):207–14.

24. Mathieu C. Minimising hypoglycaemia in the real world: the challenge of insulin. Diabetologia. 2021;64(5):978–84.

25. Foster NC, Beck RW, Miller KM, Clements MA, Rickels MR, Dimeglio LA, et al. State of Type 1 Diabetes Management and Outcomes from the T1D Exchange in 2016-2018. Diabetes Technol Ther. 2019;21(2):66–72.

26. Chatwin H, Broadley M, Speight J, Cantrell A, Sutton A, Heller S, et al. The impact of hypoglycaemia on quality of life outcomes among adults with type 1 diabetes: A systematic review. Diabetes Res Clin Pract. 2021;174:108752.

27. Norris JM, Johnson RK, Stene LC. Type 1 diabetes—early life origins and changing epidemiology. Lancet Diabetes Endocrinol. 2020;8(3):226–38.

28. Gregory GA, Robinson TIG, Linklater SE, Wang F, Colagiuri S, de Beaufort C, et al. Global incidence, prevalence, and mortality of type 1 diabetes in 2021 with projection to 2040: a modelling study. Lancet Diabetes Endocrinol. 2022 Oct;10(10):741–60.

29. Patterson CC, Harjutsalo V, Rosenbauer J, Neu A, Cinek O, Skrivarhaug T, et al. Trends and cyclical variation in the incidence of childhood type 1 diabetes in 26 European centres in the 25 year period 1989–2013: a multicentre prospective registration study. Diabetologia. 2019;62(3):408–17.

30. Patterson CC, Dahlquist GG, Gyürüs E, Green A, Soltész G, Schober E, et al. Incidence trends for childhood type 1 diabetes in Europe during 1989-2003 and predicted new cases 2005-20: a multicentre prospective registration study. Lancet. 2009;373(9680):2027–33.

31. Chobot A, Polanska J, Brandt A, Deja G, Glowinska-Olszewska B, Pilecki O, et al. Updated 24-year trend of Type 1 diabetes incidence in children in Poland reveals a sinusoidal pattern and sustained increase. Diabet Med. 2017;34(9):1252–8.

32. Berhan Y, Waernbaum I, Lind T, Möllsten A, Dahlquist G. Thirty years of prospective nationwide incidence of childhood type 1 diabetes: The accelerating increase by time tends to level off in Sweden. Diabetes.

2011;60(2):577–81.

33. Manuwald U, Schoffer O, Kugler J, Riemenschneider H, Kapellen TM, Kiess W, et al. Trends in incidence and prevalence of type 1 diabetes between 1999 and 2019 based on the Childhood Diabetes Registry of Saxony, Germany. PLoS One. 2021;16(12 December):1–10.

34. Waernbaum I, Lind T, Möllsten A, Dahlquist G. The incidence of childhood-onset type 1 diabetes, time trends and association with the population composition in Sweden: a 40 year follow-up. Diabetologia. 2022 Oct 20;1–8.

35. Krischer JP, Liu X, Lernmark Å, Hagopian WA, Rewers MJ, She JX, et al. The influence of type 1 diabetes genetic susceptibility regions, age, sex, and family history on the progression from multiple autoantibodies to type 1 diabetes: A teddy study report. Diabetes. 2017;66(12):3122–9.

36. Verbeeten KC, Elks CE, Daneman D, Ong KK. Association between childhood obesity and subsequent Type 1 diabetes: A systematic review and meta-analysis. Vol. 28, Diabetic Medicine. 2011. p. 10–8.

37. Oikarinen S, Tauriainen S, Hober D, Lucas B, Vazeou A, Sioofy-Khojine A, et al. Virus antibody survey in different european populations indicates risk association between coxsackievirus B1 and type 1 diabetes. Diabetes. 2014;63(2):655–62.

38. Vehik K, Lynch KF, Wong MC, Tian X, Ross MC, Gibbs RA, et al. Prospective virome analyses in young children at increased genetic risk for type 1 diabetes. Nat Med. 2019 Dec 1;25(12):1865–72.

39. Söderström U, Åman J, Hjern A. Being born in Sweden increases the risk for type 1 diabetes - A study of migration of children to Sweden as a natural experiment. Acta Paediatr Int J Paediatr. 2012 Jan;101(1):73–7.

40. Raymond NT, Jones JR, Swift PGF, Davies MJ, Lawrence IG, McNally PG, et al. Comparative incidence of type I diabetes in children aged under 15 years from South Asian and white or other ethnic backgrounds in Leicestershire, UK, 1989 to 1998. Diabetologia. 2001;44(SUPPL. 3).

41. Kondrashova A, Reunanen A, Romanov A, Karvonen A, Viskari H, Vesikari T, et al. A six-fold gradient in the incidence of type 1 diabetes at the eastern border of Finland. Ann Med. 2005;37(1):67–72.

42. Gillespie KM, Bain SC, Barnett PAH, Bingley PPJ, Christie MR, Gill G V., et al. The rising incidence of childhood type 1 diabetes and reduced contribution

of high-risk HLA haplotypes. Lancet. 2004 Nov 6;364(9446):1699–700.

43. Fourlanos S, Varney MD, Tait BD, Morahan G, Honeyman MC, Colman PG, et al. The rising incidence of type 1 diabetes is accounted for by cases with lower-risk human leukocyte antigen genotypes. Diabetes Care. 2008 Aug;31(8):1546–9.

44. Redondo MJ, Steck AK, Pugliese A. Genetics of type 1 diabetes. Pediatr Diabetes. 2018 May 1;19(3):346–53.

45. Primavera M, Giannini C, Chiarelli F. Prediction and Prevention of Type 1 Diabetes. Front Endocrinol (Lausanne). 2020;11(June):1–9.

46. Redondo MJ, Steck AK, Pugliese A. Genetics of type 1 diabetes. Pediatr Diabetes. 2018 May 1;19(3):346–53.

47. Pang H, Lin J, Luo S, Huang G, Li X, Xie Z, et al. The missing heritability in type 1 diabetes. Diabetes, Obes Metab. 2022 Oct 1;24(10):1901–11.

48. Qu HQ, Qu J, Bradfield J, Marchand L, Glessner J, Chang X, et al. Genetic architecture of type 1 diabetes with low genetic risk score informed by 41 unreported loci. Commun Biol. 2021 Dec 1;4(1).

49. Pang H, Xia Y, Luo S, Huang G, Li X, Xie Z, et al. Emerging roles of rare and low-frequency genetic variants in type 1 diabetes mellitus. J Med Genet. 2021 May 1;58(5):289–96.

50. Polychronakos C, Li Q. Understanding type 1 diabetes through genetics: Advances and prospects. Nat Rev Genet. 2011 Oct 18;12(11):781–92.

51. Tapia G, Mårild K, Dahl SR, Lund-Blix NA, Viken MK, Lie BA, et al. Maternal and newborn Vitamin D–binding protein, Vitamin D levels, Vitamin D receptor genotype, and childhood type 1 diabetes. Diabetes Care. 2019 Apr 1;42(4):553–9.

52. Norris JM, Lee HS, Frederiksen B, Erlund I, Uusitalo U, Yang J, et al. Plasma 25-Hydroxyvitamin D concentration and risk of islet autoimmunity. Diabetes. 2018 Jan 1;67(1):146–54.

53. Sahin GS, Lee H, Engin F. An accomplice more than a mere victim: The impact of β-cell ER stress on type 1 diabetes pathogenesis. Mol Metab. 2021 Dec 1;54:101365.

54. Rodriguez-Calvo T, Chen Y-C, Verchere CB, Haataja L, Arvan P, Leete P, et al. Altered β-Cell Prohormone Processing and Secretion in Type 1 Diabetes. Diabetes. 2021 May 1;70(5):1038–50.

55. Roep BO, Thomaidou S, van Tienhoven R, Zaldumbide A. Type 1 diabetes mellitus as a disease of the β-cell (do not blame the immune system?). Nat Rev Endocrinol. 2021 Mar 1;17(3):150–61.

56. Mallone R, Eizirik DL. Presumption of innocence for beta cells: why are they vulnerable autoimmune targets in type 1 diabetes? Diabetologia. 2020 Oct 7;63(10):1999–2006.

57. Redondo MJ, Jeffrey J, Fain PR, Eisenbarth GS, Orban T. Concordance for Islet Autoimmunity among Monozygotic Twins. N Engl J Med. 2008 Dec 25;359(26):2849–50.

58. Ferrat LA, Vehik K, Sharp SA, Lernmark Å, Rewers MJ, She JX, et al. A combined risk score enhances prediction of type 1 diabetes among susceptible children. Nat Med 2020 268. 2020 Aug 7;26(8):1247–55.

59. Redondo MJ, Concannon P. Genetics of type 1 diabetes comes of age. Diabetes Care. 2020 Jan 1;43(1):16–8.

60. Nerup J, Platz P, Andersen OO, Christy M, Lyngs o e J, Poulsen JE, et al. HL-A ANTIGENS AND DIABETES MELLITUS. Lancet. 1974 Oct 12;304(7885):864–6.

61. Roep BO, Thomaidou S, van Tienhoven R, Zaldumbide A. Type 1 diabetes mellitus as a disease of the β-cell (do not blame the immune system?). Nat Rev Endocrinol. 2021 Dec 8;17(3):150–61.

62. Onengut-Gumuscu S, Chen WM, Burren O, Cooper NJ, Quinlan AR, Mychaleckyj JC, et al. Fine mapping of type 1 diabetes susceptibility loci and evidence for colocalization of causal variants with lymphoid gene enhancers. Nat Genet. 2015 Mar 9;47(4):381–6.

63. Barrett JC, Clayton DG, Concannon P, Akolkar B, Cooper JD, Erlich HA, et al. Genome-wide association study and meta-analysis find that over 40 loci affect risk of type 1 diabetes. Nat Genet. 2009 May 10;41(6):703–7.

64. Noble JA. Immunogenetics of type 1 diabetes: A comprehensive review. J Autoimmun. 2015 Nov 1;64:101–12.

65. Grant SFA, Wells AD, Rich SS. Next steps in the identification of gene targets for type 1 diabetes. Diabetologia. 2020 Aug 14;63(11):2260–9.

66. Bonifacio E, Beyerlein A, Hippich M, Winkler C, Vehik K, Weedon MN, et al. Genetic scores to stratify risk of developing multiple islet autoantibodies and type 1 diabetes: A prospective study in children. PLoS Med. 2018 Apr

1;15(4):e1002548.

67. Sharp SA, Rich SS, Wood AR, Jones SE, Beaumont RN, Harrison JW, et al. Development and standardization of an improved type 1 diabetes genetic risk score for use in newborn screening and incident diagnosis. Diabetes Care. 2019 Feb 1;42(2):200–7.

68. Ziegler AG, Achenbach P, Berner R, Casteels K, Danne T, Gündert M, et al. Oral insulin therapy for primary prevention of type 1 diabetes in infants with high genetic risk: The GPPAD-POInT (global platform for the prevention of autoimmune diabetes primary oral insulin trial) study protocol. BMJ Open. 2019 Jun 1;9(6):e028578.

69. Besser REJ, Bell KJ, Couper JJ, Ziegler A-G, Wherrett DK, Knip M, et al. <scp>ISPAD</scp> Clinical Practice Consensus Guidelines 2022: Stages of type 1 diabetes in children and adolescents. Pediatr Diabetes. 2022 Dec 30;23(8):1175–87.

70. Hekkala AM, Ilonen J, Toppari J, Knip M, Veijola R. Ketoacidosis at diagnosis of type 1 diabetes: Effect of prospective studies with newborn genetic screening and follow up of risk children. Pediatr Diabetes. 2018 Mar;19(2):314–9.

71. Redondo MJ, Geyer S, Steck AK, Sharp S, Wentworth JM, Weedon MN, et al. A type 1 diabetes genetic risk score predicts progression of islet autoimmunity and development of type 1 diabetes in individuals at risk. Diabetes Care. 2018;41(9):1887–94.

72. Carr ALJ, Perry DJ, Lynam AL, Chamala S, Flaxman CS, Sharp SA, et al. Histological validation of a type 1 diabetes clinical diagnostic model for classification of diabetes. Diabet Med. 2020 Dec 1;37(12):2160–8.

73. Oram RA, Sharp SA, Pihoker C, Ferrat L, Imperatore G, Williams A, et al. Utility of Diabetes Type–Specific Genetic Risk Scores for the Classification of Diabetes Type Among Multiethnic Youth. Diabetes Care. 2022 Mar 21;45(5):1124–31.

74. Kent SC, Mannering SI, Michels AW, Babon JAB. Deciphering the Pathogenesis of Human Type 1 Diabetes (T1D) by Interrogating T Cells from the “Scene of the Crime.” Curr Diab Rep. 2017 Sep 2;17(10):1–11.

75. Lindley S, Dayan CM, Bishop A, Roep BO, Peatman M, Tree TIM. Defective suppressor function in CD4+CD25+ T-cells from patients with type 1 diabetes. Diabetes. 2005 Jan 1;54(1):92–9.

76. Bloem SJ, Roep BO. The elusive role of B lymphocytes and islet autoantibodies in (human) type 1 diabetes. Diabetologia. 2017 Jul 1;60(7):1185–9.

77. Culina S, Lalanne AI, Afonso G, Cerosaletti K, Pinto S, Sebastiani G, et al. Islet-reactive CD8+ T cell frequencies in the pancreas, but not in blood, distinguish type 1 diabetic patients from healthy donors. Sci Immunol. 2018 Feb 2;3(20).

78. Babon JAB, Denicola ME, Blodgett DM, Crèvecoeur I, Buttrick TS, Maehr R, et al. Analysis of self-antigen specificity of islet-infiltrating T cells from human donors with type 1 diabetes. Nat Med. 2016 Oct 31;22(12):1482–7.

79. Lampeter EF, Homberg M, Gries FA, Kolb H, Quabeck K, Schaefer UW, et al. Transfer of insulin-dependent diabetes between HLA-identical siblings by bone marrow transplantation. Lancet. 1993 May 15;341(8855):1243–4.

80. Ziegler AG, Rewers M, Simell O, Simell T, Lempainen J, Steck A, et al. Seroconversion to multiple islet autoantibodies and risk of progression to diabetes in children. JAMA. 2013 Jun 19;309(23):2473–9.

81. Yu W, Jiang N, Ebert PJR, Kidd BA, Müller S, Lund PJ, et al. Clonal Deletion Prunes but Does Not Eliminate Self-Specific αβ CD8+ T Lymphocytes. Immunity. 2015 May 19;42(5):929–41.

82. Gonzalez-Duque S, Azoury ME, Colli ML, Afonso G, Turatsinze JV, Nigi L, et al. Conventional and Neo-antigenic Peptides Presented by β Cells Are Targeted by Circulating Naïve CD8+ T Cells in Type 1 Diabetic and Healthy Donors. Cell Metab. 2018 Dec 4;28(6):946-960.e6.

83. Bender C, Rodriguez-Calvo T, Amirian N, Coppieters KT, von Herrath MG. The healthy exocrine pancreas contains preproinsulin-specific CD8 T cells that attack islets in type 1 diabetes. Sci Adv. 2020;6(42):1–9.

84. Coppieters KT, Dotta F, Amirian N, Campbell PD, Kay TWH, Atkinson MA, et al. Demonstration of islet-autoreactive CD8 T cells in insulitic lesions from recent onset and long-term type 1 diabetes patients. J Exp Med. 2012 Jan;209(1):51–60.

85. Campbell-Thompson M, Fu A, Kaddis JS, Wasserfall C, Schatz DA, Pugliese A, et al. Insulitis and β-cell mass in the natural history of type 1 diabetes. Diabetes. 2016 Mar 1;65(3):719–31.

86. Mallone R, Eizirik DL. Presumption of innocence for beta cells: why are they vulnerable autoimmune targets in type 1 diabetes? Diabetologia.

2020;63(10):1999–2006.

87. Leete P, Mallone R, Richardson SJ, Sosenko JM, Redondo MJ, Evans-Molina C. The Effect of Age on the Progression and Severity of Type 1 Diabetes: Potential Effects on Disease Mechanisms. Curr Diab Rep. 2018;18(11).

88. Bottazzo GF. Death of a beta cell: Homicide or suicide? Diabet Med. 1986 Mar 1;3(2):119–30.

89. Eizirik DL, Miani M, Cardozo AK. Signalling danger: Endoplasmic reticulum stress and the unfolded protein response in pancreatic islet inflammation. Diabetologia. 2013 Feb 7;56(2):234–41.

90. Meyerovich K, Ortis F, Allagnat F, Cardozo AK. Endoplasmic reticulum stress and the unfolded protein response in pancreatic islet inflammation. J Mol Endocrinol. 2016 Jul 1;57(1):R1–17.

91. Miki A, Ricordi C, Sakuma Y, Yamamoto T, Misawa R, Mita A, et al. Divergent antioxidant capacity of human islet cell subsets: A potential cause of beta-cell vulnerability in diabetes and islet transplantation. PLoS One. 2018 May 1;13(5):e0196570.

92. Ifie E, Russell MA, Dhayal S, Leete P, Sebastiani G, Nigi L, et al. Unexpected subcellular distribution of a specific isoform of the Coxsackie and adenovirus receptor, CAR-SIV, in human pancreatic beta cells. Diabetologia. 2018 Nov 1;61(11):2344–55.

93. Morgan NG, Richardson SJ. Fifty years of pancreatic islet pathology in human type 1 diabetes: insights gained and progress made. Diabetologia. 2018;61(12):2499–506.

94. Quesada-Masachs E, Zilberman S, Rajendran S, Chu T, McArdle S, Kiosses WB, et al. Upregulation of HLA class II in pancreatic beta cells from organ donors with type 1 diabetes. Diabetologia. 2022;65(2):387–401.

95. Roep BO, Kleijwegt FS, Van Halteren AGS, Bonato V, Boggi U, Vendrame F, et al. Islet inflammation and CXCL10 in recent-onset type 1 diabetes. Clin Exp Immunol. 2010 Mar 1;159(3):338–43.

96. Marhfour I, Lopez XM, Lefkaditis D, Salmon I, Allagnat F, Richardson SJ, et al. Expression of endoplasmic reticulum stress markers in the islets of patients with type 1 diabetes. Diabetologia. 2012 Sep 15;55(9):2417–20.

97. Kim YK, Sussel L, Davidson HW. Inherent beta cell dysfunction contributes to autoimmune susceptibility. Biomolecules. 2021 Mar 30;11(4):512.

98. Muralidharan C, Conteh AM, Marasco MR, Crowder JJ, Kuipers J, de Boer P, et al. Pancreatic beta cell autophagy is impaired in type 1 diabetes. Diabetologia. 2021;64(4):865–77.

99. Rodriguez-Calvo T, Chen YC, Verchere CB, Haataja L, Arvan P, Leete P, et al. Altered β-Cell Prohormone Processing and Secretion in Type 1 Diabetes. Diabetes. 2021 May 1;70(5):1038–50.

100. Rodriguez-Calvo T, Zapardiel-Gonzalo J, Amirian N, Castillo E, Lajevardi Y, Krogvold L, et al. Increase in pancreatic proinsulin and preservation of β-cell mass in autoantibody-positive donors prior to type 1 diabetes onset. Diabetes. 2017 May 1;66(5):1334–45.

101. Vecchio F, Messina G, Giovenzana A, Petrelli A. New Evidence of Exocrine Pancreatopathy in Pre-symptomatic and Symptomatic Type 1 Diabetes. Curr Diab Rep. 2019 Aug 31;19(10):1–8.

102. Nigi L, Maccora C, Dotta F, Sebastiani G. From immunohistological to anatomical alterations of human pancreas in type 1 diabetes: New concepts on the stage. Vol. 36, Diabetes/Metabolism Research and Reviews. John Wiley and Sons Ltd; 2020. p. e3264.

103. Atkinson MA, Campbell-Thompson M, Kusmartseva I, Kaestner KH. Organisation of the human pancreas in health and in diabetes. Diabetologia. 2020;63(10):1966–73.

104. Gepts W. Pathologic anatomy of the pancreas in juvenile diabetes mellitus. Diabetes. 1965;14(10):619–33.

105. Foulis AK, Stewart JA. The pancreas in recent-onset Type 1 (insulin-dependent) diabetes mellitus: insulin content of islets, insulitis and associated changes in the exocrine acinar tissue. Diabetologia. 1984 Jun;26(6):456–61.

106. Foster TP, Bruggeman B, Campbell-Thompson M, Atkinson MA, Haller MJ, Schatz DA. Exocrine pancreas dysfunction in type 1 diabetes. Endocr Pract. 2020 Dec 1;26(12):1505–13.

107. Alexandre-Heymann L, Mallone R, Boitard C, Scharfmann R, Larger E. Structure and function of the exocrine pancreas in patients with type 1 diabetes. Vol. 20, Reviews in Endocrine and Metabolic Disorders. Springer New York LLC; 2019. p. 129–49.

108. Nigi L, Maccora C, Dotta F, Sebastiani G. From immunohistological to anatomical alterations of human pancreas in type 1 diabetes: New

concepts on the stage. Diabetes Metab Res Rev. 2020;36(4):1–18.

109. Mohapatra S, Majumder S, Smyrk TC, Zhang L, Matveyenko A, Kudva YC, et al. Diabetes Mellitus is Associated with an Exocrine Pancreatopathy: Conclusions from a Review of Literature. Pancreas. 2016 Sep 1;45(8):1104–10.

110. Campbell-Thompson ML, Filipp SL, Grajo JR, Nambam B, Beegle R, Middlebrooks EH, et al. Relative pancreas volume is reduced in first-degree relatives of patients with type 1 diabetes. Diabetes Care. 2019;42(2):281–7.

111. Wright JJ, Saunders DC, Dai C, Poffenberger G, Cairns B, Serreze D V., et al. Decreased pancreatic acinar cell number in type 1 diabetes. Diabetologia. 2020 Jul 9;63(7):1418–23.

112. Tang X, Kusmartseva I, Kulkarni S, Posgai A, Speier S, Schatz DA, et al. Image-Based Machine Learning Algorithms for Disease Characterization in the Human Type 1 Diabetes Pancreas. Am J Pathol. 2021 Dec;191(3):454–62.

113. Bonnet-Serrano F, Diedisheim M, Mallone R, Larger E. Decreased α-cell mass and early structural alterations of the exocrine pancreas in patients with type 1 diabetes: An analysis based on the nPOD repository. PLoS One. 2018 Jan 1;13(1).

114. Almaça J, Weitz J, Rodriguez-Diaz R, Pereira E, Caicedo A. The Pericyte of the Pancreatic Islet Regulates Capillary Diameter and Local Blood Flow. Cell Metab. 2018 Mar 6;27(3):630-644.e4.

115. Rowe P, Wasserfall C, Croker B, Campbell-Thompson M, Pugliese A, Atkinson M, et al. Increased complement activation in human type 1 diabetes pancreata. Diabetes Care. 2013 Nov;36(11):3815–7.

116. Granlund L, Hedin A, Wahlhütter M, Seiron P, Korsgren O, Skog O, et al. Histological and transcriptional characterization of the pancreatic acinar tissue in type 1 diabetes. BMJ Open Diabetes Res Care. 2021 May 24;9(1).

117. de Boer P, Pirozzi NM, Wolters AHG, Kuipers J, Kusmartseva I, Atkinson MA, et al. Large-scale electron microscopy database for human type 1 diabetes. Nat Commun. 2020 Dec 1;11(1):1–9.

118. Liu CW, Atkinson MA, Zhang Q. Type 1 diabetes cadaveric human pancreata exhibit a unique exocrine tissue proteomic profile. Proteomics. 2016 Apr 13;16(9):1432–46.

119. Woo J, Sudhir PR, Zhang Q. Pancreatic Tissue Proteomics Unveils Key Proteins, Pathways, and Networks Associated with Type 1 Diabetes. Vol. 14, Proteomics - Clinical Applications. 2020.

120. Välikangas T, Lietzén N, Jaakkola MK, Krogvold L, Eike MC, Kallionpää H, et al. Pancreas Whole Tissue Transcriptomics Highlights the Role of the Exocrine Pancreas in Patients With Recently Diagnosed Type 1 Diabetes. Front Endocrinol (Lausanne). 2022 Apr 13;13:550.

121. Fasolino M, Schwartz GW, Patil AR, Mongia A, Golson ML, Wang YJ, et al. Single-cell multi-omics analysis of human pancreatic islets reveals novel cellular states in type 1 diabetes. Nat Metab. 2022 Feb 28;4(2):284–99.

122. Foster TP, Bruggeman B, Campbell-Thompson M, Atkinson MA, Haller MJ, Schatz DA. Exocrine pancreas dysfunction in type 1 diabetes. Vol. 26, Endocrine Practice. 2020. p. 1505–13.

123. Dozio N, Indirli R, Giamporcaro GM, Frosio L, Mandelli A, Laurenzi A, et al. Impaired exocrine pancreatic function in different stages of type 1 diabetes. BMJ Open Diabetes Res Care. 2021;9(1):1–7.

124. Giovenzana A, Vecchio F, Cugnata F, Nonis A, Mandelli A, Stabilini A, et al. Exocrine pancreas function is impaired in adult relatives of patients with type 1 diabetes. Acta Diabetol. 2022 Apr 1;59(4):473–9.

125. Vecchio F, Messina G, Giovenzana A, Petrelli A. New Evidence of Exocrine Pancreatopathy in Pre-symptomatic and Symptomatic Type 1 Diabetes. Vol. 19, Current Diabetes Reports. Current Medicine Group LLC 1; 2019.

126. Di Cesare E, Previti M, Lombardo F, Mazzù N, Di Benedetto A, Cucinotta D. Prevalence of autoantibodies to carbonic anhydrase II and lactoferrin in patients with type 1 diabetes. In: Annals of the New York Academy of Sciences. John Wiley & Sons, Ltd; 2004. p. 131–2.

127. Hardt PD, Ewald N, Bröckling K, Tanaka S, Endo T, Kloer HU, et al. Distinct autoantibodies against exocrine pancreatic antigens in European patients with type 1 diabetes mellitus and non-alcoholic chronic pancreatitis. Vol. 9, Journal of the Pancreas. 2008. p. 683–9.

128. Panicot L, Mas E, Thivolet C, Lombardo D. Circulating antibodies against an exocrine pancreatic enzyme in type 1 diabetes. Diabetes. 1999 Dec 1;48(12):2316–23.

129. Ross JJ, Wasserfall CH, Bacher R, Perry DJ, McGrail K, Posgai AL, et al. Exocrine Pancreatic Enzymes Are a Serological Biomarker for Type 1

Diabetes Staging and Pancreas Size. Diabetes. 2021 Apr 1;70(4):944–54.

130. Chiou J, Geusz RJ, Okino ML, Han JY, Miller M, Melton R, et al. Interpreting type 1 diabetes risk with genetics and single-cell epigenomics. Nature. 2021 May 19;594(7863):398–402.

131. Richardson SJ, Pugliese A. Pancreas pathology in type 1 diabetes: an evolving story. J Endocrinol. 2022;252(2):R41–57.

132. Lernmark Å. Etiology of Autoimmune Islet Disease: Timing Is Everything. Diabetes. 2021 Jul 1;70(7):1431–9.

133. Rodriguez-Calvo T, Ekwall O, Amirian N, Zapardiel-Gonzalo J, Von Herrath MG. Increased immune cell infiltration of the exocrine pancreas: A possible contribution to the pathogenesis of type 1 diabetes. Diabetes. 2014 Nov 1;63(11):3880–90.

134. Sims EK, DiMeglio LA. Cause or effect? A review of clinical data demonstrating beta cell dysfunction prior to the clinical onset of type 1 diabetes. Mol Metab. 2019 Sep 1;27:S129–38.

135. Evans-Molina C, Sims EK, DiMeglio LA, Ismail HM, Steck AK, Palmer JP, et al. β Cell dysfunction exists more than 5 years before type 1 diabetes diagnosis. JCI Insight. 2018 Aug 9;3(15):0–10.

136. Sosenko JM, Skyler JS, Beam CA, Krischer JP, Greenbaum CJ, Mahon J, et al. Acceleration of the loss of the first-phase insulin response during the progression to type 1 diabetes in diabetes prevention trial-type 1 participants. Diabetes. 2013 Dec;62(12):4179–83.

137. Bogun MM, Bundy BN, Goland RS, Greenbaum CJ. C-peptide levels in subjects followed longitudinally before and after type 1 diabetes diagnosis in trialnet. Diabetes Care. 2020 Aug 1;43(8):1836–42.

138. Linsley PS, Greenbaum CJ, Nepom GT. Uncovering pathways to personalized therapies in type 1 diabetes. Diabetes. 2021 Apr 1;70(4):831–41.

139. Fonolleda M, Murillo M, Vázquez F, Bel J, Vives-Pi M. Remission Phase in Paediatric Type 1 Diabetes: New Understanding and Emerging Biomarkers. Horm Res Paediatr. 2017;88(5):307–15.

140. Powers AC. Type 1 diabetes mellitus: much progress, many opportunities. J Clin Invest. 2021 Apr 15;131(8).

141. Association AD. 2. Classification and diagnosis of diabetes: Standards of

medical care in diabetes-2021. Diabetes Care. 2021 Jan 1;44(Supplement_1):S15–33.

142. Jones AG, Hattersley AT. The clinical utility of C-peptide measurement in the care of patients with diabetes. Diabet Med. 2013 Jul 1;30(7):803–17.

143. McLaughlin KA, Richardson CC, Ravishankar A, Brigatti C, Liberati D, Lampasona V, et al. Identification of tetraspanin-7 as a target of autoantibodies in type 1 diabetes. Diabetes. 2016 Jun 1;65(6):1690–8.

144. Thomas NJ, Jones SE, Weedon MN, Shields BM, Oram RA, Hattersley AT. Frequency and phenotype of type 1 diabetes in the first six decades of life: a cross-sectional, genetically stratified survival analysis from UK Biobank. Lancet Diabetes Endocrinol. 2018 Feb 1;6(2):122–9.

145. Samuelsson U, Lindblad B, Carlsson A, Forsander G, Ivarsson S, Kockum I, et al. Residual beta cell function at diagnosis of type 1 diabetes in children and adolescents varies with gender and season. Diabetes Metab Res Rev. 2013 Jan 1;29(1):85–9.

146. Shi M, Xie Y, Tang R, Zhong T, Zhou Z, Li X. Three-phasic pattern of C-peptide decline in type 1 diabetes patients with partial remission. Diabetes Metab Res Rev. 2021 Nov 7;37(8).

147. Lötvall J, Akdis CA, Bacharier LB, Bjermer L, Casale TB, Custovic A, et al. Asthma endotypes: A new approach to classification of disease entities within the asthma syndrome. J Allergy Clin Immunol. 2011 Feb;127(2):355–60.

148. Oram RA, Jones AG, Besser REJ, Knight BA, Shields BM, Brown RJ, et al. The majority of patients with long-duration type 1 diabetes are insulin microsecretors and have functioning beta cells. Diabetologia. 2014 Oct 12;57(1):187–91.

149. Krischer JP, Lynch KF, Schatz DA, Ilonen J, Lernmark Å, Hagopian WA, et al. The 6 year incidence of diabetes-associated autoantibodies in genetically at-risk children: the TEDDY study. Diabetologia. 2015 May 1;58(5):980–7.

150. Vehik K, Bonifacio E, Lernmark Å, Yu L, Williams A, Schatz D, et al. Hierarchical order of distinct autoantibody spreading and progression to type 1 diabetes in the Teddy study. Diabetes Care. 2020 Sep 1;43(9):2066–73.

151. Ilonen J, Laine AP, Kiviniemi M, Härkönen T, Lempainen J, Knip M. Associations between deduced first islet specific autoantibody with sex,

age at diagnosis and genetic risk factors in young children with type 1 diabetes. Pediatr Diabetes. 2022 Sep 1;23(6):693–702.

152. Laine AP, Valta M, Toppari J, Knip M, Veijola R, Ilonen J, et al. Non-HLA Gene Polymorphisms in the Pathogenesis of Type 1 Diabetes: Phase and Endotype Specific Effects. Front Immunol. 2022 Jun 21;13:3009.

153. Kwon BC, Anand V, Achenbach P, Dunne JL, Hagopian W, Hu J, et al. Progression of type 1 diabetes from latency to symptomatic disease is predicted by distinct autoimmune trajectories. Nat Commun. 2022 Mar 21;13(1):1–9.

154. Krischer JP, Liu X, Lernmark Å, Hagopian WA, Rewers MJ, She J-X, et al. Characteristics of children diagnosed with type 1 diabetes before vs after 6 years of age in the TEDDY cohort study. Diabetologia. 2021 Oct 22;64(10):2247–57.

155. Kwon BC, Achenbach P, Anand V, Frohnert BI, Hagopian W, Hu J, et al. Islet Autoantibody Levels Differentiate Progression Trajectories in Individuals with Presymptomatic Type 1 Diabetes. Diabetes. 2022 Sep 16;

156. So M, Speake C, Steck AK, Lundgren M, Colman PG, Palmer JP, et al. Advances in Type 1 Diabetes Prediction Using Islet Autoantibodies: Beyond a Simple Count. Endocr Rev. 2021 Sep 28;42(5):584–604.

157. Vecchio F, Buono N Lo, Stabilini A, Nigi L, Dufort MJ, Geyer S, et al. Abnormal neutrophil signature in the blood and pancreas of presymptomatic and symptomatic type 1 diabetes Federica Vecchio , … , the Type 1 Diabetes TrialNet Study Group , Manuela Battaglia Find the latest version : Abnormal neutrophil signature in the bl. JCI Insight. 2018;3(18):1–17.

158. So M, Elso CM, Tresoldi E, Pakusch M, Pathiraj V, Wentworth JM, et al. Proinsulin C-peptide is an autoantigen in people with type 1 diabetes. Proc Natl Acad Sci U S A. 2018 Oct 16;115(42):10732–7.

159. Sims EK, Chaudhry Z, Watkins R, Syed F, Blum J, Ouyang F, et al. Elevations in the fasting serum Proinsulin-to-C-peptide ratio precede the onset of type 1 diabetes. Diabetes Care. 2016;39(9):1519–26.

160. Arif S, Leete P, Nguyen V, Marks K, Nor NM, Estorninho M, et al. Blood and Islet Phenotypes Indicate Immunological Heterogeneity in Type 1 Diabetes. Diabetes. 2014 Nov 1;63(11):3835–45.

161. Leete P, Oram RA, McDonald TJ, Shields BM, Ziller C, Roep BO, et al.

Studies of insulin and proinsulin in pancreas and serum support the existence of aetiopathological endotypes of type 1 diabetes associated with age at diagnosis. Diabetologia. 2020;63(6):1258–67.

162. Sims EK, Syed F, Nyalwidhe J, Bahnson HT, Haataja L, Speake C, et al. Abnormalities in proinsulin processing in islets from individuals with longstanding T1D. Transl Res. 2019;213:90–9.

163. Parviainen A, Härkönen T, Ilonen J, But A, Knip M. Heterogeneity of Type 1 Diabetes at Diagnosis Supports Existence of Age-Related Endotypes. Diabetes Care. 2022 Apr 1;45(4):871–9.

164. Inshaw JRJ, Cutler AJ, Crouch DJM, Wicker LS, Todd JA. Genetic variants predisposing most strongly to type 1 diabetes diagnosed under age 7 years lie near candidate genes that function in the immune system and in pancreatic B-cells. Diabetes Care. 2020 Jan 1;43(1):169–77.

165. Dufort MJ, Greenbaum CJ, Speake C, Linsley PS. Cell type-specific immune phenotypes predict loss of insulin secretion in new-onset type 1 diabetes. JCI insight. 2019 Feb 21;4(4).

166. Hao W, Gitelman S, Di Meglio LA, Boulware D, Greenbaum CJ. Fall in C-peptide during first 4 years from diagnosis of type 1 diabetes: Variable relation to age, HbA1c, and insulin dose. Diabetes Care. 2016;39(10):1664–70.

167. Wiedeman AE, Muir VS, Rosasco MG, DeBerg HA, Presnell S, Haas B, et al. Autoreactive CD8+ T cell exhaustion distinguishes subjects with slow type 1 diabetes progression. J Clin Invest. 2020 Jan 2;130(1):480–90.

168. Pollé OG, Delfosse A, Martin M, Louis J, Gies I, den Brinker M, et al. Glycemic Variability Patterns Strongly Correlate With Partial Remission Status in Children With Newly Diagnosed Type 1 Diabetes. Diabetes Care. 2022;45(October):2360–8.

169. Deligne C, You S, Mallone R. Personalized Immunotherapies for Type 1 Diabetes: Who, What, When, and How? J Pers Med. 2022;12(4):542.

170. Achenbach P, Hippich M, Zapardiel-Gonzalo J, Karges B, Holl RW, Petrera A, et al. A classification and regression tree analysis identifies subgroups of childhood type 1 diabetes. eBioMedicine. 2022;82:104118.

171. Mortensen HB, Hougaard P, Swift P, Hansen L, Holl RW, Hoey H, et al. New definition for the partial remission period in children and adolescents with type 1 diabetes. Diabetes Care. 2009;32(8):1384–90.

172. Chen Y, Xia Y, Xie Z, Zhong T, Tang R, Li X, et al. The Unfavorable Impact of DR9/DR9 Genotype on the Frequency and Quality of Partial Remission in Type 1 Diabetes. J Clin Endocrinol Metab. 2022 Jan 1;107(1):E293–302.

173. Lundberg RL, Marino KR, Jasrotia A, Maranda LS, Barton BA, Alonso LC, et al. Partial clinical remission in type 1 diabetes: a comparison of the accuracy of total daily dose of insulin of <0.3 units/kg/day to the gold standard insulin-dose adjusted hemoglobin A1c of ≤9 for the detection of partial clinical remission. J Pediatr Endocrinol Metab. 2017;30(8):823–30.

174. Lombardo F, Valenzise M, Wasniewska M, Messina MF, Ruggeri C, Arrigo T, et al. Two-year prospective evaluation of the factors affecting honeymoon frequency and duration in children with insulin dependent diabetes mellitus: the key-role of age at diagnosis. Diabetes Nutr Metab. 2002 Aug;15(4):246–51.

175. Nielens N, Pollé O, Robert A, Lysy PA. Integration of Routine Parameters of Glycemic Variability in a Simple Screening Method for Partial Remission in Children with Type 1 Diabetes. J Diabetes Res. 2018;2018.

176. Mayer-Davis EJ, Kahkoska AR, Jefferies C, Dabelea D, Balde N, Gong CX, et al. ISPAD Clinical Practice Consensus Guidelines 2018: Definition, epidemiology, and classification of diabetes in children and adolescents. Pediatr Diabetes. 2018;19:7–19.

177. Nagl K, Hermann JM, Plamper M, Schröder C, Dost A, Kordonouri O, et al. Factors contributing to partial remission in type 1 diabetes: analysis based on the insulin dose-adjusted HbA1c in 3657 children and adolescents from Germany and Austria. Pediatr Diabetes. 2017 Sep 1;18(6):428–34.

178. Max Andersen MLC, Hougaard P, Pörksen S, Nielsen LB, Fredheim S, Svensson J, et al. Partial Remission Definition: Validation based on the insulin dose-adjusted HbA1c (IDAA1C) in 129 Danish Children with New-Onset Type 1 Diabetes. Pediatr Diabetes. 2014;15(7):469–76.

179. Mørk FCB, Madsen JOB, Jensen AK, Hall G V., Pilgaard KA, Pociot F, et al. Differences in insulin sensitivity in the partial remission phase of childhood type 1 diabetes; a longitudinal cohort study. Diabet Med. 2022 Feb 1;39(2):e14702.

180. Nwosu BU. Partial Clinical Remission of Type 1 Diabetes Mellitus in Children: Clinical Applications and Challenges with its Definitions. Eur Med journal Diabetes. 2019 Mar;4(1):89.

181. Schölin A, Nyström L, Arnqvist H, Bolinder J, Björk E, Berne C, et al. Proinsulin/C-peptide ratio, glucagon and remission in new-onset Type 1 diabetes mellitus in young adults. Diabet Med. 2011 Feb 1;28(2):156–61.

182. Kaas A, Max Andersen ML, Fredheim S, Hougaard P, Buschard K, Petersen JS, et al. Proinsulin, GLP-1, and glucagon are associated with partial remission in children and adolescents with newly diagnosed type 1 diabetes. Pediatr Diabetes. 2012 Feb 1;13(1):51–8.

183. Li X, Zhong T, Tang R, Wu C, Xie Y, Liu F, et al. PD-1 and PD-L1 Expression in Peripheral CD4/CD8+ T Cells Is Restored in the Partial Remission Phase in Type 1 Diabetes. J Clin Endocrinol Metab. 2020 Jun 1;105(6):1947–56.

184. Gomez-Muñoz L, Perna-Barrull D, Caroz-Armayones JM, Murillo M, Rodriguez-Fernandez S, Valls A, et al. Candidate Biomarkers for the Prediction and Monitoring of Partial Remission in Pediatric Type 1 Diabetes. Front Immunol. 2022 Feb 23;13:555.

185. Fitas AL, Martins C, Borrego LM, Lopes L, Jörns A, Lenzen S, et al. Immune cell and cytokine patterns in children with type 1 diabetes mellitus undergoing a remission phase: A longitudinal study. Pediatr Diabetes. 2018 Aug 1;19(5):963–71.

186. Villalba A, Fonolleda M, Murillo M, Rodriguez-Fernandez S, Ampudia R-M, Perna-Barrull D, et al. Partial remission and early stages of pediatric type 1 diabetes display immunoregulatory changes. A pilot study. Transl Res. 2019 Aug 1;210:8–25.

187. Pfleger C, Mortensen HB, Hansen L, Herder C, Roep BO, Hoey H, et al. Association of IL-1ra and adiponectin with C-peptide and remission in patients with type 1 diabetes. Diabetes. 2008 Apr;57(4):929–37.

188. Spijker HS, Ravelli RBG, Mommaas-Kienhuis AM, Van Apeldoorn AA, Engelse MA, Zaldumbide A, et al. Conversion of Mature Human β-Cells into Glucagon- Producing α-Cells. Diabetes. 2013 Jul 1;62(7):2471–80.

189. Moin ASM, Butler AE. Alterations in Beta Cell Identity in Type 1 and Type 2 Diabetes. Curr Diab Rep. 2019 Sep 1;19(9):1–12.

190. Nasteska D, Viloria K, Everett L, Hodson DJ. Informing β-cell regeneration strategies using studies of heterogeneity. Mol Metab. 2019 Sep 1;27(Suppl):S49–59.

191. Yazidi M, Mahjoubi S, Oueslati I, Chaker F, Chihaoui M. The remission phase in adolescents and young adults with newly diagnosed type 1

diabetes mellitus: prevalence, predicting factors and glycemic control during follow-up. Arch Endocrinol Metab. 2022;66(2).

192. Niedzwiecki P, Pilacinski S, Uruska A, Zozulinska-Ziolkiewicz D. Clinical remission of type 1 diabetes predicts higher insulin sensitivity at 7 years from diagnosis of the disease. Diabetes Technol Ther. 2020 Jul 27;22(8):577–83.

193. Niedzwiecki P, Pilacinski S, Uruska A, Adamska A, Naskret D, Zozulinska-Ziolkiewicz D. Influence of remission and its duration on development of early microvascular complications in young adults with type 1 diabetes. J Diabetes Complications. 2015 Nov;29(8):1105–11.

194. Nwosu BU, Rupendu S, Zitek-Morrison E, Patel D, Villalobos-Ortiz TR, Jasmin G, et al. Pubertal Lipid Levels Are Significantly Lower in Youth With Type 1 Diabetes Who Experienced Partial Clinical Remission. J Endocr Soc. 2019 Apr 1;3(4):737–47.

195. Tang R, Zhong T, Wu C, Zhou Z, Li X. The Remission Phase in Type 1 Diabetes: Role of Hyperglycemia Rectification in Immune Modulation. Front Endocrinol (Lausanne). 2019 Dec 3;10.

196. Testa R, Bonfigli AR, Prattichizzo F, La Sala L, De Nigris V, Ceriello A. The “Metabolic Memory” Theory and the Early Treatment of Hyperglycemia in Prevention of Diabetic Complications. Nutrients. 2017 Apr 28;9(5).

197. Cimbek EA, Bozklr A, Usta D, Beyhun NE, Ökten A, Karagüzel G. Partial remission in children and adolescents with type 1 diabetes: An analysis based on the insulin dose-adjusted hemoglobin A1c. J Pediatr Endocrinol Metab. 2021 Oct 1;34(10):1311–7.

198. Mazarello Paes V, Charalampopoulos D, Edge J, Taylor-Robinson D, Stephenson T, Amin R. Predictors of glycemic control in the first year of diagnosis of childhood onset type 1 diabetes: A systematic review of quantitative evidence. Pediatr Diabetes. 2018 Feb 1;19(1):18–26.

199. Zhong T, Tang R, Xie Y, Liu F, Li X, Zhou Z. Frequency, clinical characteristics, and determinants of partial remission in type 1 diabetes: Different patterns in children and adults. J Diabetes. 2020;

200. Jamiołkowska-Sztabkowska M, Głowińska-Olszewska B, Łuczyński W, Konstantynowicz J, Bossowski A. Regular physical activity as a physiological factor contributing to extend partial remission time in children with new onset diabetes mellitus—Two years observation. Pediatr Diabetes.

2020;21(5):800–7.

201. Marino KR, Lundberg RL, Jasrotia A, Maranda LS, Thompson MJ, Barton BA, et al. A predictive model for lack of partial clinical remission in new-onset pediatric type 1 diabetes. PLoS One. 2017;12(5):e0176860.

202. Zhong T, Tang R, Gong S, Li J, Li X, Zhou Z. The remission phase in type 1 diabetes: Changing epidemiology, definitions, and emerging immuno-metabolic mechanisms. Diabetes Metab Res Rev. 2020;36(2):0–2.

203. Nwosu BU. Partial Clinical Remission of Type 1 Diabetes: The Need for an Integrated Functional Definition Based on Insulin-Dose Adjusted A1c and Insulin Sensitivity Score. Front Endocrinol (Lausanne). 2022;13:683.

204. Sperling MA, Laffel LM. Current Management of Glycemia in Children with Type 1 Diabetes Mellitus. Solomon CG, editor. N Engl J Med. 2022 Mar 23;386(12):1155–64.

205. Arnqvist HJ, Westerlund MC, Fredrikson M, Ludvigsson J, Nordwall M. Impact of HbA1c Followed 32 Years From Diagnosis of Type 1 Diabetes on Development of Severe Retinopathy and Nephropathy: The VISS Study. Diabetes Care. 2022;

206. Chan JCN, Lim LL, Wareham NJ, Shaw JE, Orchard TJ, Zhang P, et al. The Lancet Commission on diabetes: using data to transform diabetes care and patient lives. Lancet. 2020 Dec 19;396(10267):2019–82.

207. Nathan DM. The diabetes control and complications trial/epidemiology of diabetes interventions and complications study at 30 years: Overview. Diabetes Care. 2014;37(1):9–16.

208. Sönksen PH, Judd SL, Lowy C. HOME MONITORING OF BLOOD-GLUCOSE. Method for Improving Diabetic Control. Lancet. 1978;311(8067):729–32.

209. Pamungkas RA, Chamroonsawasdi K. Psychological problems related to capillary blood glucose testing and insulin injection among diabetes patients. Front Nurs. 2020;7(2):87–95.

210. Evans M, Welsh Z, Ells S, Seibold A. The Impact of Flash Glucose Monitoring on Glycaemic Control as Measured by HbA1c: A Meta-analysis of Clinical Trials and Real-World Observational Studies. Diabetes Ther. 2020;11(1):83–95.

211. Laffel LM, Kanapka LG, Beck RW, Bergamo K, Clements MA, Criego A, et al. Effect of Continuous Glucose Monitoring on Glycemic Control in

Adolescents and Young Adults with Type 1 Diabetes: A Randomized Clinical Trial. JAMA - J Am Med Assoc. 2020;323(23):2388–96.

212. Strategies to Enhance New CGM Use in Early Childhood (SENCE) Study Group. A Randomized Clinical Trial Assessing Continuous Glucose Monitoring (CGM) Use With Standardized Education With or Without a Family Behavioral Intervention Compared With Fingerstick Blood Glucose Monitoring in Very Young Children With Type 1 Diabetes. Diabetes Care. 2021;44(2):464–72.

213. Charleer S, De Block C, Nobels F, Radermecker RP, Lowyck I, Mullens A, et al. Sustained impact of real-time continuous glucose monitoring in adults with type 1 diabetes on insulin pump therapy: Results after the 24-month rescue study. Diabetes Care. 2020;43(12):3016–23.

214. Beck RW, Bergenstal RM, Laffel LM, Pickup JC. Advances in technology for management of type 1 diabetes. Vol. 394, The Lancet. 2019. p. 1265–73.

215. Xu Y, Grimsmann JM, Karges B, Hofer S, Danne T, Holl RW, et al. Personal Glycation Factors and Calculated Hemoglobin A1c for Diabetes Management: Real-World Data from the Diabetes Prospective Follow-up (DPV) Registry. Diabetes Technol Ther. 2021 Jun 1;23(6):452–9.

216. Stene LC, Hyöty H. A novel approach to the investigation of potential precipitating factors in type 1 diabetes. Vol. 7, Pediatric Diabetes. 2006. p. 143–5.

217. Choudhary A, Adhikari S, White PC. Impact of the COVID-19 pandemic on management of children and adolescents with Type 1 diabetes. BMC Pediatr. 2022;22(1):1–7.

218. Danne T, Lanzinger S, De Bock M, Rhodes ET, Alonso GT, Barat P, et al. A Worldwide Perspective on COVID-19 and Diabetes Management in 22,820 Children from the SWEET Project: Diabetic Ketoacidosis Rates Increase and Glycemic Control Is Maintained. Diabetes Technol Ther. 2021;23(9):632–41.

219. Boyle KD, Keyes-Elstein L, Ehlers MR, McNamara J, Rigby MR, Gitelman SE, et al. Two- and Four-Hour tests differ in capture of C-peptide responses to a mixed meal in type 1 diabetes. Diabetes Care. 2016;39(6):e76–8.

220. Ruan Y, Willemsen RH, Wilinska ME, Tauschmann M, Dunger DB, Hovorka R. Mixed-meal tolerance test to assess residual beta-cell secretion: Beyond the area-under-curve of plasma C-peptide concentration. Pediatr Diabetes.

2019 May 1;20(3):282–5.

221. Carr ALJ, Oram RA, Marren SM, McDonald TJ, Narendran P, Andrews RC. Measurement of Peak C-Peptide at Diagnosis Informs Glycemic Control but not Hypoglycemia in Adults with Type 1 Diabetes. J Endocr Soc. 2021;5(10):1–10.

222. Oram RA, Sims EK, Evans-Molina C. Beta cells in type 1 diabetes: mass and function; sleeping or dead? Diabetologia. 2019;62(4):567–77.

223. Kontola H, Alanko I, Koskenniemi JJ, Löyttyniemi E, Itoshima S, Knip M, et al. Exploring Minimally Invasive Approach to Define Stages of Type 1 Diabetes Remotely. Diabetes Technol Ther. 2022;24(9):655–65.

224. Warncke K, Weiss A, Achenbach P, von dem Berge T, Berner R, Casteels K, et al. Elevations in blood glucose before and after the appearance of islet autoantibodies in children. J Clin Invest. 2022;132(20).

225. Benedict C, Vogel H, Jonas W, Woting A, Blaut M, Schürmann A, et al. Gut microbiota and glucometabolic alterations in response to recurrent partial sleep deprivation in normal-weight young individuals. Mol Metab. 2016;5(12):1175–86.

226. Rewers M, She JX, Ziegler AG, Simell OG, Lernmark Å, Hagopian WA, et al. The environmental determinants of diabetes in the young (TEDDY) study. Ann N Y Acad Sci. 2008 Dec;1150(1):1–13.

227. Dayan CM, Korah M, Tatovic D, Bundy BN, Herold KC. Changing the landscape for type 1 diabetes: the first step to prevention. Lancet. 2019;394(10205):1286–96.

228. Bonifacio E, Weiß A, Winkler C, Hippich M, Rewers MJ, Toppari J, et al. An Age-Related Exponential Decline in the Risk of Multiple Islet Autoantibody Seroconversion During Childhood. Diabetes Care. 2021 Oct 1;44(10):2260–8.

229. Dayan CM, Besser REJ, Oram RA, Hagopian W, Vatish M, Bendor-Samuel O, et al. Preventing type 1 diabetes in childhood. Science (80-). 2021 Jul 30;373(6554):506–10.

230. Smith LB, Liu X, Johnson SB, Tamura R, Elding Larsson H, Ahmed S, et al. Family adjustment to diabetes diagnosis in children: Can participation in a study on type 1 diabetes genetic risk be helpful? Pediatr Diabetes. 2018;19(5):1025–33.

231. Ziegler AG, Kick K, Bonifacio E, Haupt F, Hippich M, Dunstheimer D, et al. Yield of a Public Health Screening of Children for Islet Autoantibodies in Bavaria, Germany. JAMA - J Am Med Assoc. 2020;323(4):339–51.

232. Lundgren M, Jonsdottir B, Elding Larsson H. Effect of screening for type 1 diabetes on early metabolic control: the DiPiS study. Diabetologia. 2019;62(1):53–7.

233. Duca LM, Wang B, Rewers M, Rewers A. Diabetic ketoacidosis at diagnosis of type 1 diabetes predicts poor long-term glycemic control. Diabetes Care. 2017;40(9):1249–55.

234. Sims EK, Besser REJ, Dayan C, Rasmussen CG, Greenbaum C, Griffin KJ, et al. Screening for Type 1 Diabetes in the General Population: A Status Report and Perspective. Diabetes. 2022;71(4):610–23.

235. McQueen RB, Rasmussen CG, Waugh K, Frohnert BI, Steck AK, Yu L, et al. Cost and cost-effectiveness of large-scale screening for type 1 diabetes in Colorado. Diabetes Care. 2020;43(7):1496–503.

236. Besser REJ, Ng SM, Gregory JW, Dayan CM, Randell T, Barrett T. General population screening for childhood type 1 diabetes: is it time for a UK strategy? Arch Dis Child. 2022;107(9):790–5.

237. Chiarelli F, Rewers M, Phillip M. Screening of Islet Autoantibodies for Children in the General Population: a Position Statement endorsed by the European Society for Pediatric Endocrinology. Horm Res Paediatr. 2022;95(4).

238. Atkinson MA, Roep BO, Posgai A, Wheeler DCS, Peakman M. The challenge of modulating β-cell autoimmunity in type 1 diabetes. Lancet Diabetes Endocrinol. 2019;7(1):52–64.

239. Roep BO, Montero E, van Tienhoven R, Atkinson MA, Schatz DA, Mathieu C. Defining a cure for type 1 diabetes: a call to action. Lancet Diabetes Endocrinol. 2021 Sep 1;9(9):553–5.

240. von Scholten BJ, Kreiner FF, Gough SCL, von Herrath M. Current and future therapies for type 1 diabetes. Diabetologia. 2021 Feb 17;64(5):1037–48.

241. Herold KC, Bundy BN, Alice Long S, Bluestone JA, DiMeglio LA, Dufort MJ, et al. An anti-CD3 antibody, teplizumab, in relatives at risk for type 1 diabetes. N Engl J Med. 2019;381(7):603–13.

242. Quattrin T, Haller MJ, Steck AK, Felner EI, Li Y, Xia Y, et al. Golimumab and

Beta-Cell Function in Youth with New-Onset Type 1 Diabetes. N Engl J Med. 2020;383(21):2007–17.

243. Bundy BN, Krischer JP. A quantitative measure of treatment response in recent-onset type 1 diabetes. Endocrinol Diabetes Metab. 2020;3(3):e00143.

244. Carr ALJ, Evans-Molina C, Oram RA. Precision medicine in type 1 diabetes. Diabetologia. 2022 Nov 22;65(11):1854–66.

245. Battaglia M, Buckner JH, Levings MK, Richardson SJ, Wong FS, Tree TI. Identifying the 'Achilles heel' of type 1 diabetes. Clin Exp Immunol. 2021 Apr 23;204(2):167–78.

246. Matthews JB, Staeva TP, Bernstein PL, Peakman M, Von Herrath M. Developing combination immunotherapies for type 1 diabetes: Recommendations from the ITN-JDRF Type 1 Diabetes Combination Therapy Assessment Group. Clin Exp Immunol. 2010;160(2):176–84.

247. Warshauer JT, Bluestone JA, Anderson MS. New Frontiers in the Treatment of Type 1 Diabetes. Cell Metab. 2020;31(1):46–61.

248. Purohit S, Sharma A, Hopkins D, Steed L, Bode B, Anderson SW, et al. Large-scale discovery and validation studies demonstrate significant reductions in circulating levels of IL8, IL-1Ra, MCP-1, and MIP-1β in patients with type 1 diabetes. J Clin Endocrinol Metab. 2015;100(9):E1179–87.

249. Sims EK, Bahnson HT, Nyalwidhe J, Haataja L, Davis AK, Speake C, et al. Proinsulin secretion is a persistent feature of type 1 diabetes. In: Diabetes Care. 2019. p. 258–64.

250. Crèvecoeur I, Vig S, Mathieu C, Overbergh L. Understanding type 1 diabetes through proteomics. Expert Rev Proteomics. 2017;14(7):571–80.

251. Xhonneux LP, Knight O, Lernmark Å, Bonifacio E, Hagopian WA, Rewers MJ, et al. Transcriptional networks in at-risk individuals identify signatures of type 1 diabetes progression. Sci Transl Med. 2021;13(587).

252. Bahreini F, Rayzan E, Rezaei N. MicroRNAs and Diabetes Mellitus Type 1. Curr Diabetes Rev. 2022 Feb 16;18(2).

253. Mitchell AM, Baschal EE, McDaniel KA, Simmons KM, Pyle L, Waugh K, et al. Temporal development of T cell receptor repertoires during childhood in health and disease. JCI insight. 2022;7(18).

254. Alcazar O, Hernandez LF, Nakayasu ES, Nicora CD, Ansong C, Muehlbauer MJ, et al. Parallel multi-omics in high-risk subjects for the identification of integrated biomarker signatures of type 1 diabetes. Biomolecules. 2021;11(3):1–25.

255. Kahkoska AR, Adair LA, Aiello AE, Burger KS, Buse JB, Crandell J, et al. Identification of clinically relevant dysglycemia phenotypes based on continuous glucose monitoring data from youth with type 1 diabetes and elevated hemoglobin A1c. Pediatr Diabetes. 2019;20(5):556–66.

256. MAP-T1D [Internet]. [cited 2022 Oct 3]. Available from: https://www.map-t1d.com/

257. Jansen TJP, Brom M, Boss M, Buitinga M, Tack CJ, van Meijel LA, et al. Importance of beta cell mass for glycaemic control in people with type 1 diabetes. Diabetologia. 2022;

258. Colinet V, Lysy PA. Characterization of Post-Hypoglycemic Hyperglycemia in Children and Adolescents With Type 1 Diabetes: The EPHICA Study. Front Endocrinol (Lausanne). 2022;13:1225.

259. Danne T, Phillip M, Buckingham BA, Jarosz-Chobot P, Saboo B, Urakami T, et al. ISPAD Clinical Practice Consensus Guidelines 2018: Insulin treatment in children and adolescents with diabetes. Pediatr Diabetes. 2018 Oct;19:115–35.

260. Martin S, Pawlowski B, Greulich B, Ziegler AG, Mandrup-Poulsen T, Mahon J. Natural course of remission in IDDM during 1st yr after diagnosis. Diabetes Care. 1992;15(1):66–74.

261. Pecheur A, Barrea T, Vandooren V, Beauloye V, Robert A, Lysy PA. Characteristics and Determinants of Partial Remission in Children with Type 1 Diabetes Using the Insulin-Dose-Adjusted A1C Definition. J Diabetes Res. 2014;2014:1–7.

262. Abdul-Rasoul M, Habib H, Al-Khouly M. “The honeymoon phase” in children with type 1 diabetes mellitus: Frequency, duration, and influential factors. Pediatr Diabetes. 2006;7(2):101–7.

263. Bowden SA. Partial Remission (honeymoon phase) in Type 1 Diabetes Mellitus. Front Clin Drug Res - Diabetes Obes. 2017;4(August):1–20.

264. Kara Ö, Esen İ, Tepe D. Factors influencing frequency and duration of remission in children and adolescents newly diagnosed with type 1 diabetes. Med Sci Monit. 2018;24:5996–6001.

265. Schölin A, Törn C, Nyström L, Berne C, Arnqvist H, Blohmé G, et al. Normal weight promotes remission and low number of islet antibodies prolong the duration of remission in Type 1 diabetes. Diabet Med. 2004;21(5):447–55.

266. Chobot A, Stompór J, Szyda K, Sokołowska M, Deja G, Polańska J, et al. Remission phase in children diagnosed with type 1 diabetes in years 2012 to 2013 in Silesia, Poland: An observational study. Pediatr Diabetes. 2019;20(3):286–92.

267. Wentworth JM, Bediaga NG, Giles LC, Ehlers M, Gitelman SE, Geyer S, et al. Beta cell function in type 1 diabetes determined from clinical and fasting biochemical variables. Diabetologia. 2019;62(1):33–40.

268. Oram RA, McDonald TJ, Shields BM, Hudson MM, Shepherd MH, Hammersley S, et al. Most people with long-duration type 1 diabetes in a large population-based study are insulin microsecretors. Diabetes Care. 2015;38(2):323–8.

269. Kuhtreiber WM, Washer SLL, Hsu E, Zhao M, Reinhold P, Burger D, et al. Low levels of C-peptide have clinical significance for established Type 1 diabetes. Diabet Med. 2015;32(10):1346–53.

270. Keenan HA, Sun JK, Levine J, Doria A, Aiello LP, Eisenbarth G, et al. Residual insulin production and pancreatic β-cell turnover after 50 years of diabetes: Joslin medalist study. Diabetes. 2010;59(11):2846–53.

271. Gibb FW, McKnight JA, Clarke C, Strachan MWJ. Preserved C-peptide secretion is associated with fewer low-glucose events and lower glucose variability on flash glucose monitoring in adults with type 1 diabetes. Diabetologia. 2020;63(5):906–14.

272. Rickels MR, Evans-Molina C, Bahnson HT, Ylescupidez A, Nadeau KJ, Hao W, et al. High residual C-peptide likely contributes to glycemic control in type 1 diabetes. J Clin Invest. 2020;130(4):1850–62.

273. Jeyam A, Colhoun H, McGurnaghan S, Blackbourn L, McDonald TJ, Palmer CNA, et al. Clinical Impact of Residual C-Peptide Secretion in Type 1 Diabetes on Glycemia and Microvascular Complications. Diabetes Care. 2021;44(2):390–8.

274. Nwosu BU, Zhang B, Ayyoub SS, Choi S, Villalobos-Ortiz TR, Alonso LC, et al. Children with type 1 diabetes who experienced a honeymoon phase had significantly lower LDL cholesterol 5 years after diagnosis. Pietropaolo M, editor. PLoS One. 2018 May 16;13(5):e0196912.

275. Dunger DB, Bruggraber SFA, Mander AP, Marcovecchio ML, Tree T, Chmura PJ, et al. INNODIA Master Protocol for the evaluation of investigational medicinal products in children, adolescents and adults with newly diagnosed type 1 diabetes. Trials. 2022;23(1):1–9.

276. Posevitz-Fejfár A, Posevitz V, Gross CC, Bhatia U, Kurth F, Schütte V, et al. Effects of blood transportation on human peripheral mononuclear cell yield, phenotype and function: Implications for immune cell biobanking. PLoS One. 2014;9(12):e115920.

277. Jacobsen LM, Newby BN, Perry DJ, Posgai AL, Haller MJ, Brusko TM. Immune Mechanisms and Pathways Targeted in Type 1 Diabetes. Vol. 18, Current Diabetes Reports. 2018. p. 1–20.

278. Ovalle F, Grimes T, Xu G, Patel AJ, Grayson TB, Thielen LA, et al. Verapamil and beta cell function in adults with recent-onset type 1 diabetes. Vol. 24, Nature Medicine. 2018.

279. Chmelova H, Cohrs CM, Chouinard JA, Petzold C, Kuhn M, Chen C, et al. Distinct roles of β-Cell mass and function during type 1 diabetes onset and remission. Diabetes. 2015;64(6):2148–60.

280. Monnier L, Colette C, Wojtusciszyn A, Dejager S, Renard E, Molinari N, et al. Toward defining the threshold between low and high glucose variability in diabetes. Diabetes Care. 2017;40(7):832–8.

281. Ceriello A, Novials A, Ortega E, La Sala L, Pujadas G, Testa R, et al. Evidence that hyperglycemia after recovery from hypoglycemia worsens endothelial function and increases oxidative stress and inflammation in healthy control subjects and subjects with type 1 diabetes. Diabetes. 2012;61(11):2993–7.

282. Bowden SA, Duck MM, Hoffman RP. Young children (< 5 yr) and adolescents (> 12 yr) with type 1 diabetes mellitus have low rate of partial remission: Diabetic ketoacidosis is an important risk factor. Pediatr Diabetes. 2008;9(3 PART 1):197–201.

283. Augustine P, Gent R, Louise J, Taranto M, Penno M, Linke R, et al. Pancreas size and exocrine function is decreased in young children with recent-onset Type 1 diabetes. Diabet Med. 2020 Aug 17;37(8):1340–3.

284. Virostko J, Williams J, Hilmes M, Bowman C, Wright JJ, Du L, et al. Pancreas volume declines during the first year after diagnosis of type 1 diabetes and exhibits altered diffusion at disease onset. Diabetes Care. 2019;42(2):248–57.

285. Sasamori H, Fukui T, Hayashi T, Yamamoto T, Ohara M, Yamamoto S, et al. Analysis of pancreatic volume in acute-onset, slowly-progressive and fulminant type 1 diabetes in a Japanese population. J Diabetes Investig. 2018;9(5):1091–9.

286. Damond N, Engler S, Zanotelli VRT, Schapiro D, Wasserfall CH, Kusmartseva I, et al. A Map of Human Type 1 Diabetes Progression by Imaging Mass Cytometry. Cell Metab. 2019;29(3):755-768.e5.

287. Gaglia JL, Harisinghani M, Aganj I, Wojtkiewicz GR, Hedgire S, Benoist C, et al. Noninvasive mapping of pancreatic inflammation in recent-onset type-1 diabetes patients. Proc Natl Acad Sci. 2015;112(7):2139–44.

288. Diedisheim M, Mallone R, Boitard C, Larger E. β-cell mass in nondiabetic autoantibody-positive subjects: An analysis based on the network for pancreatic organ donors database. J Clin Endocrinol Metab. 2016;101(4):1390–7.

289. Wang X, Misawa R, Zielinski MC, Cowen P, Jo J, Periwal V, et al. Regional Differences in Islet Distribution in the Human Pancreas - Preferential Beta-Cell Loss in the Head Region in Patients with Type 2 Diabetes. PLoS One. 2013;8(6):e67454.

290. Wu L, Nahm CB, Jamieson NB, Samra J, Clifton-Bligh R, Mittal A, et al. Risk factors for development of diabetes mellitus (Type 3c) after partial pancreatectomy: A systematic review. Clin Endocrinol (Oxf). 2020;92(5):396–406.

291. Hempel S, Oehme F, Ehehalt F, Solimena M, Kolbinger FR, Bogner A, et al. The Impact of Pancreatic Head Resection on Blood Glucose Homeostasis in Patients with Chronic Pancreatitis. J Clin Med. 2022;11(3):663.

292. Virostko J. Quantitative Magnetic Resonance Imaging of the Pancreas of Individuals With Diabetes. Front Endocrinol (Lausanne). 2020 Dec 4;11.

293. Virostko J, Craddock RC, Williams JM, Triolo TM, Hilmes MA, Kang H, et al. Development of a standardized MRI protocol for pancreas assessment in humans. PLoS One. 2021 Aug 1;16(8):e0256029.

294. Ho J, Mah JK, Hill MD, Pacaud D. Pediatric stroke associated with new onset type 1 diabetes mellitus: Case reports and review of the literature. Pediatr Diabetes. 2006;7(2):116–21.

295. Geyer PE, Voytik E, Treit P V, Doll S, Kleinhempel A, Niu L, et al. Plasma Proteome Profiling to detect and avoid sample-related biases in biomarker

studies. EMBO Mol Med. 2019;11(11):e10427.

296. Regnell SE, Peterson P, Trinh L, Broberg P, Leander P, Lernmark, et al. Pancreas volume and fat fraction in children with Type 1 diabetes. Diabet Med. 2016;33(10):1374–9.

297. Kaur G, Poljak A, Ali SA, Zhong L, Raftery MJ, Sachdev P. Extending the Depth of Human Plasma Proteome Coverage Using Simple Fractionation Techniques. J Proteome Res. 2021;20(2):1261–79.

298. Roger R, Hilmes MA, Williams JM, Moore DJ, Powers AC, Craddock RC, et al. Deep learning-based pancreas volume assessment in individuals with type 1 diabetes. BMC Med Imaging. 2022;22(1):1–5.

299. Margaritis K, Margioula-Siarkou G, Margioula-Siarkou C, Petousis S, Kotanidou EP, Christoforidis A, et al. Circulating serum and plasma levels of micro-RNA in type-1 diabetes in children and adolescents: a systematic review and meta-analysis. Eur J Clin Invest. 2021 Feb 9;

300. Dotta F, Ventriglia G, Snowhite I V., Pugliese A. MicroRNAs: Markers of β-cell stress and autoimmunity. Curr Opin Endocrinol Diabetes Obes. 2018;25(4):237–45.

301. Pociot F. Capturing residual beta cell function in type 1 diabetes. Diabetologia. 2019;62(1):28–32.

302. Thrailkill KM, Moreau CS, Swearingen C, Rettiganti M, Edwards K, Morales AE, et al. Insulin Pump Therapy Started at the Time of Diagnosis: Effects on Glycemic Control and Pancreatic β-Cell Function in Type 1 Diabetes. Diabetes Technol Ther. 2011;13(10):1023–30.

303. Abraham MB, Jones TW, Naranjo D, Karges B, Oduwole A, Tauschmann M, et al. ISPAD Clinical Practice Consensus Guidelines 2018: Assessment and management of hypoglycemia in children and adolescents with diabetes. Pediatr Diabetes. 2018 Oct 1;19:178–92.