Proteomic profiling with Olink® Reveal identifies potential biomarkers of diabetes and prediabetes: A case study using the Białystok PLUS Cohort

A case study performed by Olink in collaboration with Professor Karol Kaminski of the Medical University of Białystok, Poland and Dr. Anders Mälarstig of the Karolinska Institute.

Study highlights

- Plasma samples from individuals newly diagnosed with impaired glucose metabolism and healthy controls were analyzed using Olink Reveal.
- Protein levels were associated with glucose tolerance categorization, HbA1c levels, and glycemia in oral glucose tolerance tests (OGTT).
- Proteins were identified as potential diagnostic biomarkers of diabetes and prediabetes.

Background

Studies such as the UK Biobank-Pharma Proteomics Project (UKB-PPP) and consortia such as SCALLOP have highlighted the power of large-scale population health studies to identify biomarker signatures across multiple disease areas.

Biobanks that represent more focused, local populations also present significant opportunities to understand regional population dynamics due to lifestyle and genetic factors, and uncover related biological insights.

In this study, the Białystok PLUS cohort (1) —comprised of a random sampling of the population of the city of Białystok, Poland—was leveraged to identify proteins associated with type 2 diabetes (T2D) and prediabetes.

T2D accounts for 96% of diabetes cases worldwide, but the pathogenesis of the disease is incompletely understood and there is no cure (2). Prior to the development of diabetes, individuals progress through a prediabetic state, providing a window of opportunity for early detection and intervention to prevent disease progression (3).

Glucose metabolism disturbances are detected clinically via increased hemoglobin A1c (HbA1c) or hyperglycemia manifesting as impaired fasting glucose (IFG) or impaired glucose tolerance (IGT). However, such methods are not sufficient for the diagnosis of clinical consequences of prediabetes because their results are not fully proportional to the progression of the disease and organ injury, rendering their clinical prognostic power unsatisfactory (4).

In this study, Professor Karol Kaminski's group at the Medical University of Białystok, Poland and Dr. Anders Mälarstig's group at the Karolinska Institute leveraged their early access to Olink Reveal to identify protein biomarkers of T2D and prediabetes in the Białystok PLUS cohort.

Study aims

1. Compare the levels of ~1,000 proteins in plasma from individuals with newly diagnosed diabetes and prediabetes enrolled in the Białystok PLUS cohort.
2. Compare protein levels to glucose tolerance, HbA1c levels, and oral glucose tolerance tests.
3. Identify potential diagnostic protein biomarkers for diabetes, prediabetes, or disease progression.

Olink® Reveal

~1,000 robustly expressed proteins covering all major Reactome pathways

Study design

In a population group of 2,839 individuals screened in the Białystok PLUS Cohort, 508 individuals aged 35-70 years and without known diabetes mellitus or any other severe disease were selected for inclusion in this study (**Figure 1**).

Plasma was collected and diagnoses of impaired glucose metabolism were made at the time of enrollment. Glucose metabolism was assessed using HbA1c levels and oral glucose tolerance tests (OGTT). Individuals were categorized into four groups:

1. Without impaired glucose metabolism (healthy).
2. Pre-diabetic state: impaired fasting glucose (IFT).
3. Pre-diabetic state: impaired glucose tolerance (IGT).
4. Newly diagnosed diabetes (T2D; 5).

Proteomic profiling using Olink Reveal (~1,000 circulating proteins) was performed and protein levels were associated with HbA1c, OGTT, and glucose tolerance stratification of subjects.

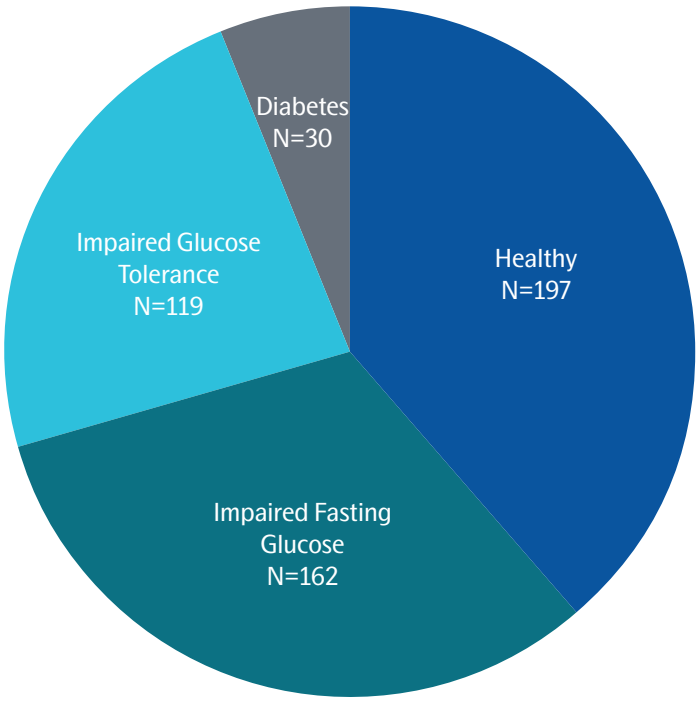


Figure 1 Overview of the study design and groups included in the analysis.

Results

Olink Reveal exhibits high detectability and precision in human plasma

~1,000 proteins were measured in healthy individuals (n=197) and those with impaired glucose metabolism (n=311). Olink Reveal exhibited a high level of detectability (mean detectability, 79%; median detectability, 99%) and precision (mean inter/intraplate CVs of 7.6% and 7.4%, respectively).

Associations were made between protein levels and three glucose metabolism tests—glucose tolerance, HbA1C levels, and glycemia in OGTT (t=0 min; 60 min, and 120 min).

22 assays were significantly associated with glucose tolerance tests, while **37 assays** were significantly associated with HbA1c levels (**Figure 2**). Those hits are now being used to develop hypotheses into the pathophysiology of impaired glucose tolerance and are the foci of ongoing investigations by Professor Karol Kaminski and Dr. Anders Mälarstig.

Olink® Reveal identifies proteins associated with impaired glucose metabolism.

37 proteins significantly associated with HbA1c levels

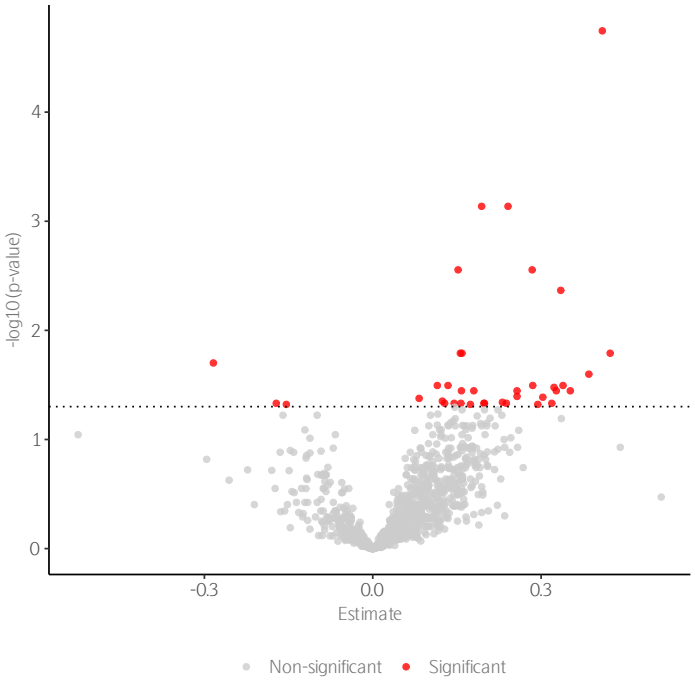
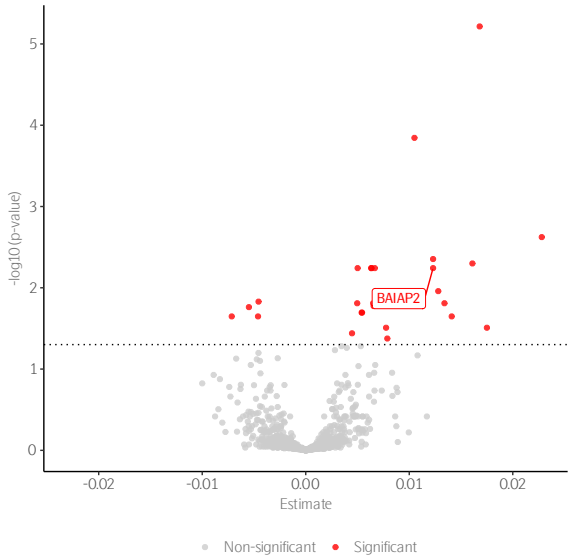


Figure 2 Volcano plot of proteins significantly associated with HbA1c levels.

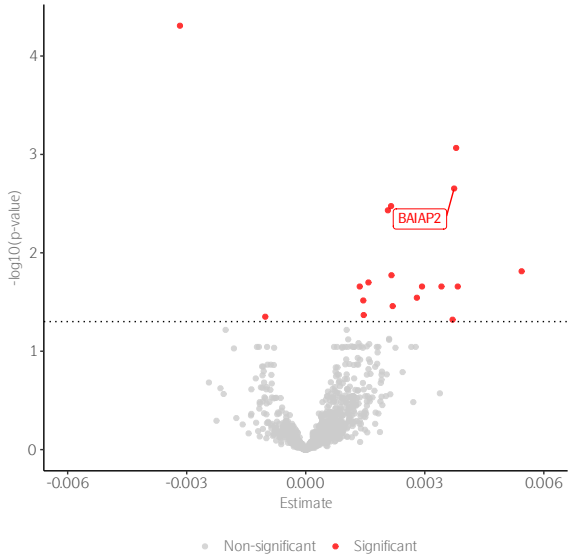
Analysis of proteins associated with OGTT time points was performed to identify those that represent potential biomarkers of diabetes and prediabetes.

38 unique proteins were associated with OGTT (all time points), with subgroup analyses identifying 28 proteins associated with OGTT at t=0 minutes, 18 associated with OGTT at t=60 minutes, and 7 associated with t=120 minutes (**Figures 3 & 4**).

Proteins associated with OGTT t=0 min



Proteins associated with OGTT t=60 min



Proteins associated with OGTT t=120 min

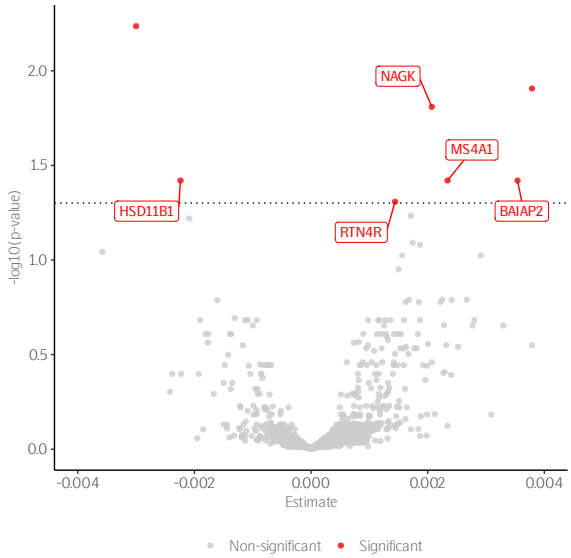


Figure 3 Volcano plots of proteins significantly associated with OGTT time points.

Number of proteins associated with OGTT time points

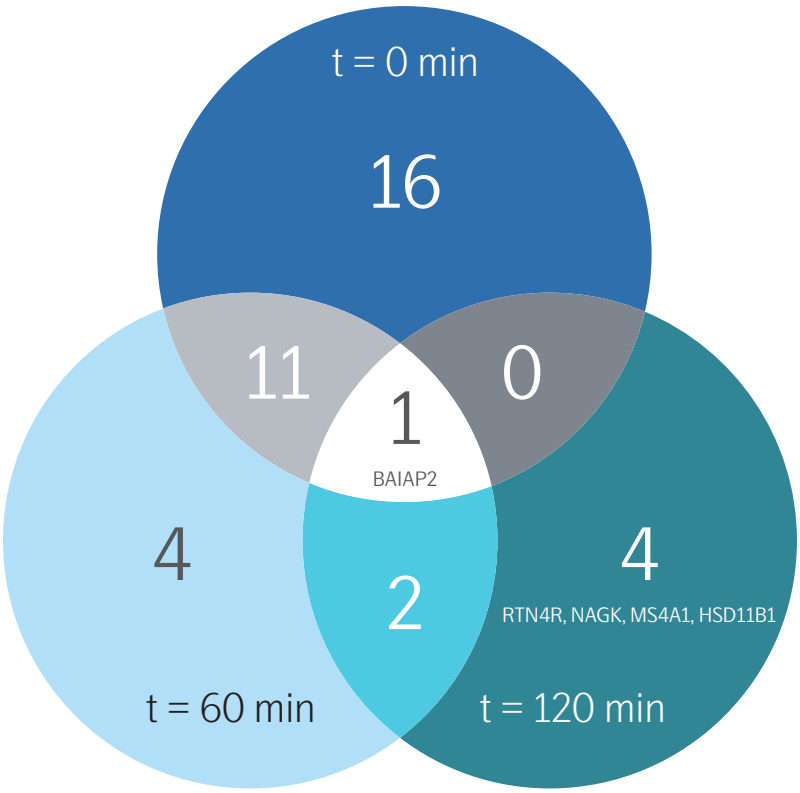


Figure 4 Venn diagram of proteins associated with OGTT at t=0 min (baseline), t=60 min, and t=120 min.

Identification of proteins potentially associated with diabetes and prediabetes

One protein, **BAIAP2**, was significantly associated with OGTT at all time points (**Figure 4**), suggesting it may be a marker of hyperglycemia. BAIAP2 is an insulin receptor tyrosine kinase substrate with a suggested role in insulin regulation.

Four proteins, including **RTN4R**, **NAGK**, **MS4A1**, and **HSD11B1** (**Figure 4**) were associated with OGTT t=120 min—but not t=0 min or t=60 min—and were considered potential biomarkers of a prediabetic state (i.e., IFG or IGT). Whether those proteins are involved in mechanisms leading to different prediabetic states is now the topic of ongoing studies.

Olink Reveal agrees with the findings of other omics studies

- RTN4R is a regulator of glucose-stimulated insulin release and suggested by other proteomic and transcriptomics studies as a diagnostic biomarker for T2D (6, 7).
- The association of NAGK with OGTT identified in this study agrees with cell culture and transcriptome studies that linked the dysregulation of NAGK to insulin resistance (8).
- Gene expression changes in MS4A1 are associated with B cell differentiation and C-peptide levels, supporting its potential as a biomarker of prediabetes or its involvement in the development of diabetes (9).
- Genetic and transcriptome studies also identified that gene expression changes and single nucleotide polymorphisms in HSD11B1 are associated with T2D and metabolic syndrome (10).

Olink Reveal enables broad profiling of all major Reactome pathways

Biological pathway analysis can yield insights into the pathophysiology of disease and reveal key metabolic processes. Olink Reveal covers all major Reactome pathways.

Multiple Reactome pathways were represented among the proteins associated with OGTT time points, including several pathways common among RTN4R, NAGK, MS4A1, and HSD11B1 (Metabolism, Metabolism of Proteins, Drug ADME), as well as those in which BAIAP2 is involved (Signal Transduction, Immune System, and Disease). The analysis of such pathways may thus serve as a starting point for follow up investigations of pathways potentially involved in prediabetic changes, diabetic states, or the pathomechanism of disease.

Conclusions

This study highlights the power of Olink Reveal to support biomarker discovery studies using local biobanks, and advance hypothesis generating activities aimed at disentangling the mechanisms of complex diseases.

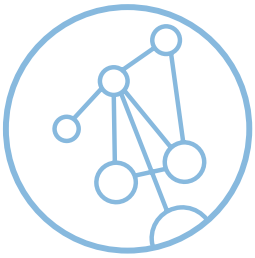
The inclusion of proteins robustly expressed in plasma covering all major Reactome pathways enabled broad profiling of biomarkers potentially involved in the development of (pre)diabetes and facilitated the generation of actionable insights for impending studies. The findings of this study have expanded the avenues of research for Professor Kaminski and Dr. Mälarstig, and have led to the generation of new hypotheses to study the pathophysiology of diabetes.

"Groups around the world are enabled to start using proteomics on their own samples. We have collected a large biobank from our local population with detailed clinical data on local healthcare priorities such as prediabetes. Olink Reveal enabled us to characterize these samples, helping us identify potential protein biomarkers that correlate with other clinical data."

Professor Karol Kaminski,
Medical University of Białystok

Olink Reveal: Accessible NGS-based proteomics

High-plex protein analysis, made accessible, flexible, and cost-effective for every lab.

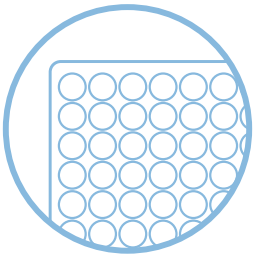


Powerful content

- Broad proteome coverage
- Deep profiling of inflammation processes

Accessible solution

- Affordable library prep per sample
- Access to proprietary data analysis tools



Simplified workflow

- Seamlessly integrated with NGS
- Efficient workflow with standard lab techniques, <2.5 hours hands on time

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