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Predictive biomarkers of rapidly developing insulin deficiency in children with type 1 diabetes

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ABSTRACT

Introduction The rate of progression to complete insulin deficiency varies greatly in type 1 diabetes. This constitutes a challenge, especially when randomizing patients in intervention trials aiming to preserve beta cell function. This study aimed to identify biomarkers predictive of either a rapid or slow disease progression in children with new-onset type 1 diabetes.

Research design and methods A retrospective, longitudinal cohort study of children (<18 years) with type 1 diabetes (N=46) was included at diagnosis and followed until complete insulinopenia (C-peptide <0.03 nmol/L). Children were grouped into rapid progressors (n=20, loss within 30 months) and slow progressors (n=26). A sex-matched control group of healthy children (N=45) of similar age was included for comparison. Multiple biomarkers were assessed by proximity extension assay (PEA) at baseline and follow-up.

Results At baseline, rapid progressors had lower C-peptide and higher autoantibody levels than slow. Three biomarkers were higher in the rapid group: carbonic anhydrase 9, corticosteroid 11-beta-dehydrogenase isozyme 1, and tumor necrosis factor receptor superfamily member 21. In a linear mixed model, 25 proteins changed over time, irrespective of group. One protein, a coxsackievirus B–adenovirus receptor (CAR) increased over time in rapid progressors. Eighty-one proteins differed between type 1 diabetes and healthy controls. Principal component analysis could not distinguish between rapid, slow, and healthy controls.

Conclusions Despite differences in individual proteins, the combination of multiple biomarkers analyzed by PEA could not distinguish the rate of progression in children with new-onset type 1 diabetes. Only one marker was altered significantly when considering both time and group effects, namely CAR, which increased significantly over time in the rapid group. Nevertheless, we did find some markers that may be useful in predicting the decline of the C-peptide. Moreover, these could potentially be important for understanding type 1 diabetes pathogenesis.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Early identification of disease progression is essential for type 1 diabetes interventional trials to preserve beta cell function and improve the reliability of study findings.

WHAT THIS STUDY ADDS

⇒ In this study of children with new-onset type 1 diabetes, three proteins distinguished rapid from slow progressors at baseline, and one protein, a coxsackievirus B-receptor, differed over time. Additionally, 81 proteins differed between patients with type 1 diabetes and healthy controls at baseline.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE, OR POLICY

⇒ Findings can help to identify rapidly progressing type 1 diabetes when stratifying patients in interventional trials and may improve the understanding of type 1 diabetes pathogenesis.

INTRODUCTION

Type 1 diabetes is caused by islet autoimmunity and declining insulin secretion. Environmental factors contribute to the condition[1] in addition to genetic predisposition.[2] Insulin depletion typically develops more rapidly in children than in adults, but the rate of progression to insulin deficiency varies greatly.

The loss of beta cell function varies with age,[3] sex,[3] genotype,[4] body mass index (BMI),[5] Haemoglobin A1c (HbA1c),[6] as well as ketoacidosis and islet cell autoantibodies at diagnosis. A residual beta cell function carries great patient benefit as even a small preserved insulin secretion is associated with fewer episodes of ketoacidosis[7] and serious hypoglycemia and less risk of late diabetes complications.[8] We have recently confirmed the association between low age, ketoacidosis, higher HbA1c, high titers of glutamic acid decarboxylase antibody (GADA) and islet antigen-2 antibody (IA-2A), and a more rapid loss of endogenous insulin secretion.[9] Moreover, we found that a rapid decline in C-peptide is associated with an increased incidence of severe hypoglycemia. Conversely, high BMI SDS (SDS=Standard deviation score, deviation of BMI from a reference population), low HbA1c during the first years, and higher

frequency of the HLADR3 genotype were associated with long-term preservation of C-peptide.[9] We have also previously found that patients with long-standing type 1 diabetes and preservation of C-peptide have increased levels of circulating interleukin (IL)-35 and a higher frequency of IL-35+ regulatory T cells (Tregs), suggestive of an altered immunological phenotype.[10]

Identifying patterns of biomarkers or individual markers that can predict disease progression rate at diagnosis could reveal novel insights regarding the pathogenesis of type 1 diabetes. Furthermore, predicting the progression rate at type 1 diabetes onset holds great value in appropriately randomizing patients to clinical intervention trials aiming to preserve beta cell function and, ideally, selecting the most appropriate beta cell preservation therapy. Therefore, we have investigated whether multiplexing of biomarkers at diagnosis can predict the rate of loss of C-peptide secretion in a cohort of children with new-onset type 1 diabetes.

RESEARCH DESIGN AND METHODS

Participant selection and study design

The study is based on a retrospective, observational study including 46 children (born between 1989 and 2007) with newly diagnosed type 1 diabetes (year of diagnosis between 2004 and 2017) who initiated insulin treatment on admission. The participants were regularly followed at the Crown Princess Victoria Children's Hospital in Linköping, Sweden. At the age of 18 years, participants were transferred to the diabetes clinic for adults. The diagnosis of type 1 diabetes was based on the criteria set by the American Diabetes Association for diagnosis and classification. At baseline, the *T1D* group was also compared with a sex-matched healthy control group of similar age, *Healthy* (N=45), from the ABIS (All Babies in Southeast Sweden) cohort.

The hypothesis was that multiplexed biomarkers can differentiate patients with rapid loss of C-peptide secretion from those with a slower decline, indicating distinct biological pathways or disease mechanisms associated with the progression of type 1 diabetes.

The aim was to investigate biomarker differences in relation to the course of residual C-peptide and whether or not multiplexed biomarkers could distinguish patients with a rapid loss of C-peptide secretion. Based on longitudinal stimulated C-peptide data, the cohort was divided into two groups, Rapid (n=20) and Slow (n=26). Rapid progression was defined as a loss of C-peptide secretion within 30 months following type 1 diabetes debut (cut-off <0.03 nmol/L). The two type 1 diabetes groups, *Rapid* and *Slow*, were also compared over time.

Descriptive data and clinical chemistry

Descriptive data of age, sex, HbA1c, blood glucose, blood pH, and C-peptide levels at the time of diagnosis before initiating insulin treatment were collected from electronic medical records. During follow-up visits (10 days, 1, 3, 9, 18, 24, and 30 months, and 3, 4, 5, and 6 years after diagnosis), additional data were recorded, including weight, height, HbA1c, and insulin dosage (expressed as units per kilogram of body weight per 24 hours). In addition, BMI and BMI SDS, adjusted for age and sex, were automatically generated using the SWEDIABKIDS register.[11]

Mixed meal tolerance tests (MMTTs) were performed under fasting conditions in the morning. Baseline measurements of C-peptide and glucose were obtained, followed by sampling at 30-minute intervals during the 120-minute test. Short-acting insulin administration was withheld for at least 6 hours before the MMTT. The composition of the mixed meal changed over time, transitioning from a standardized breakfast to a standardized liquid meal based on the participant's body weight. C-peptide concentrations were measured using a time-resolved fluoroimmunoassay with a lower detection limit of 0.03 nmol/L, and undetectable C-peptide levels were assigned a 0.01 nmol/L value for statistical analysis.

HbA1c and blood glucose measurements were performed at the Department of Clinical Chemistry, Linköping. The laboratory is certified by Swedac, a Swedish government authority. As of October 2010, HbA1c is analyzed using the International Federation of Clinical Chemistry and Laboratory Medicine reference method and expressed in mmol/mol. Before October 2010, analyses were based on the Mono S standard and expressed in percentage. HbA1c analyses performed with the Mono S standard were recalculated using the following expression: (International Federation of Clinical Chemistry and Laboratory Medicine (IFCC); mmol/mol) = 10.45 × HbA1c (Mono S; %)−10.62 (https://ngsp.org/convert1.asp).

Autoantibodies GADA (detection limit 5 IU/mL) and IA-2A (detection limit 7.5 kU/L) were analyzed using two-sided ELISA test kits from RSR (RSR, Cardiff, UK) in serum according to the instructions from the manufacturer. Samples negative for ELISA IA-2A were further analyzed with a high-sensitivity IA-2A radio binding assay. Recombinantglutamic acid decarboxylase 65 (GAD65) and islet antigen 2 (IA-2) were labeled with [^{35}S] methionine (GE Healthcare Life Sciences, Amersham, UK) by in vitro-coupled transcription and translation in the TNT SP6 coupled reticulocyte lysate system (Promega, Southampton, UK) as described. Full-length cDNA coding for human GAD65 in the pTNT vector (Promega) (pThGAD65) or the intracellular domain (aa 603–980) of IA-2 in the pSP64 Poly(A) vector (Promega) (IA-2ic) was used. These analyses were conducted at the Department of Clinical Chemistry, Skåne University Hospital, Malmö, Sweden. The intra-assay coefficient of variation for duplicates in the GADA assay was 7% and in the IA-2A 11%. In the Diabetes Autoantibody Standardization Program 2010 workshop, our laboratory was among the top-ranking laboratories for GADA in workshop sensitivity (80%) and specificity (99%) and the top-ranking

laboratory for IA-2A in workshop sensitivity (60%) and specificity (99%).[12]

Proximity extension assay (PEA)

Protein analysis was performed in undiluted EDTA plasma by multiplex PEA at Olink Proteomics AB (Uppsala, Sweden) according to the manufacturer's protocol. Two validated 92-plex panels, Olink IMMUNE ONCOLOGY (*IMO*) and Olink IMMUNE RESPONSE (*IRE*, online supplemental tables 1 and 2), were used to measure markers associated with inflammation and active immune and cytokine response. The IMO panel consists of proteins involved in tumor immunity, chemotaxis, tissue remodeling, apoptosis, cytotoxicity, metabolism, and autophagy. The IRE panel is focused on key proteins involved in adaptive immune response, viral defense, inflammation, and cytokine signaling. These panels were chosen based on their complementary immune profiles. Samples were collected and analyzed in patients with type 1 diabetes at 10 days, 3, 9, and 18 months after diagnosis. Additional samples were collected at 30, 48, and 72 months in those with remaining C-peptide at 30 months (Slow). Only baseline samples were analyzed in healthy controls.

Data analysis and statistics

Statistical analyses were performed at Olink using R V.4.1.2 (2021-11-01), apart from baseline characteristics that were analyzed in-house using RStudio V.2022.12.0+353. Values are given as means±2 SDs. P values <0.05 after correction for multiple testing with the Benjamini and Hochberg procedure (unless otherwise stated) were considered statistically significant. Samples were analyzed in three different batches. A principal component analysis (PCA) plot was used to identify outliers among 10 bridging samples to ensure accuracy. Based on this quality control (QC) plot, the *IMO* panel had no outliers, while one baseline sample in the *IRE* panel deviated from the remaining samples. This sample was hence removed from the analysis of the *IRE* panel. All samples were included in longitudinal analyses. The samples were normalized between batches using the median difference as an adjustment factor for the limit of detection. The normalization process was evaluated using density plots and was observed to make the sample distributions more similar between batches.

PCA was further used to identify the protein patterns of each group. A Welch two-sample t-test for independent samples was used to compare differences between the two groups. Analysis of variance (ANOVA) was used to compare three groups. A linear mixed-effects regression model was used to compare the *Rapid* and *Slow* groups over time. Pearson's χ^2 test was used for categorical data analyses.

Applying the recommended effect size (Cohen's f) 0.5 by Olink statistical services, a sample size of 44 in each of the two groups is required to detect differences at the 0.00027 significance level, with a power of 0.8.

RESULTS

Descriptive data of study subjects

Age at onset and sex distribution did not differ between the two groups (table 1). Group *Rapid* had a higher proportion of participants with both IA-2 and glutamic acid decarboxylase (GAD) autoantibodies (88% vs 52%, p=0.04) and lower C-peptide at diagnosis than group *Slow* (0.22 vs 0.49, p=0.01). Group *Healthy* was 45 children without autoantibodies or heredity for type 1 diabetes matched for age and sex with group *T1D*.

Proximity extension assay

Twelve non-normalized samples were excluded from this visual analysis but were otherwise included. In addition, one outlier from the *IRE* panel was excluded from all analyses (online supplemental table 3).

Table 1 Baseline characteristics

Characteristic	Rapid **(n=20)**	Slow **(n=26)**	P value
Female n (%)	13 (65)	14 (54)	0.60
Age, years	9.96 (2.29)	10.92 (2.60)	0.20
C-peptide, nmol/L	0.22 (0.12)	0.49 (0.44)	0.01*
HbA1c, mmol/mol	103.79 (19.27)	95.81 (30.95)	0.30
HbA1c%	11.67 (1.79)	10.92 (2.84)	0.30
BMI SDS, kg/m^2	−0.29 (1.02)	0.04 (1.41)	0.40
IA-2A+, n/N (%)	15/17 (88)	15/23 (65)	0.20
GADA+, n/N (%)	15/17 (88)	17/23 (74)	0.50
IA-2A+GADA+, n/N (%)	15/17 (88)	12/23 (52)	0.04*

Baseline characteristics by group. The proportion of IA-2 and GAD antibodies and C-peptide at baseline differed significantly between groups Rapid and Slow.
Significant *
BMI SDS, body mass index SD deviation score; GAD, glutamic acid decarboxylase; GADA, glutamic acid decarboxylase antibody.IA-2, islet antigen 2; IA-2A, islet antigen-2 antibody.

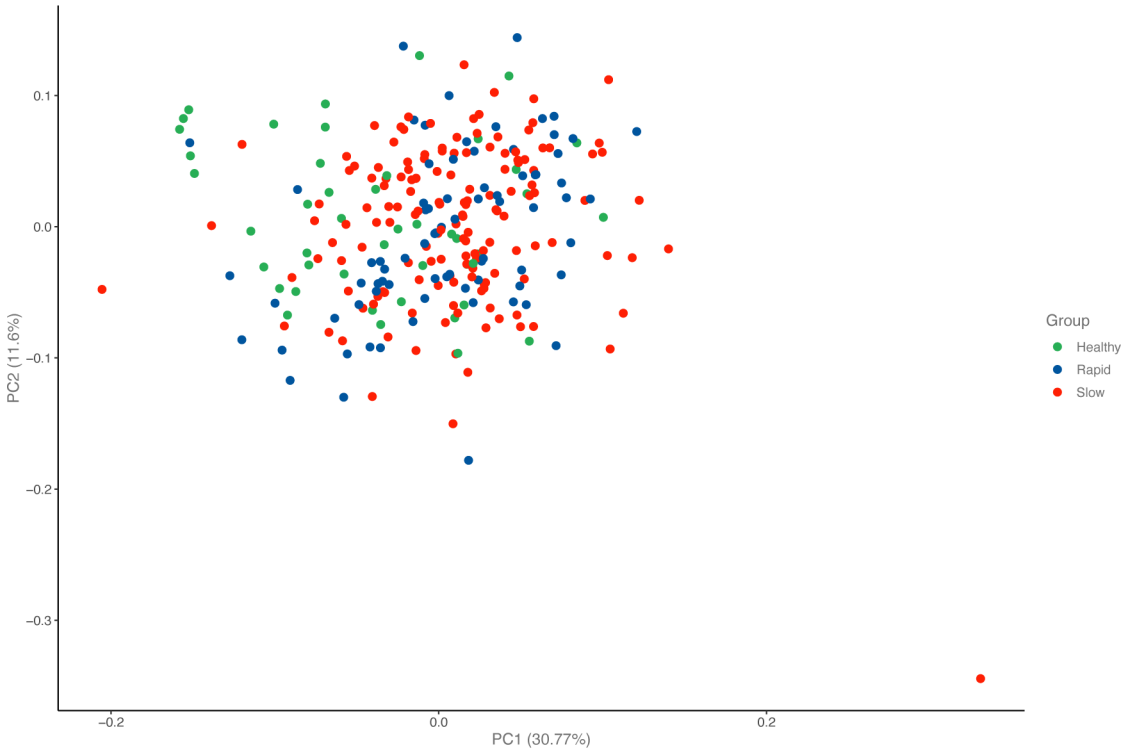

Figure 1 Principal component analysis samples in immune response panel.

Principal component analysis

At baseline, 184 biomarkers were measured in the *Rapid*, *Slow*, and *Healthy* groups. The groups had no distinctive biomarker patterns that could separate them from each other (figures 1 and 2).

Individual biomarkers

ANOVA: *Rapid* versus *Slow* versus *Healthy*

Sixty-four biomarkers differed in the ANOVA of *Rapid*, *Slow*, and *Healthy* (figure 3), with a similar outcome to the t-test findings. A post hoc analysis revealed higher

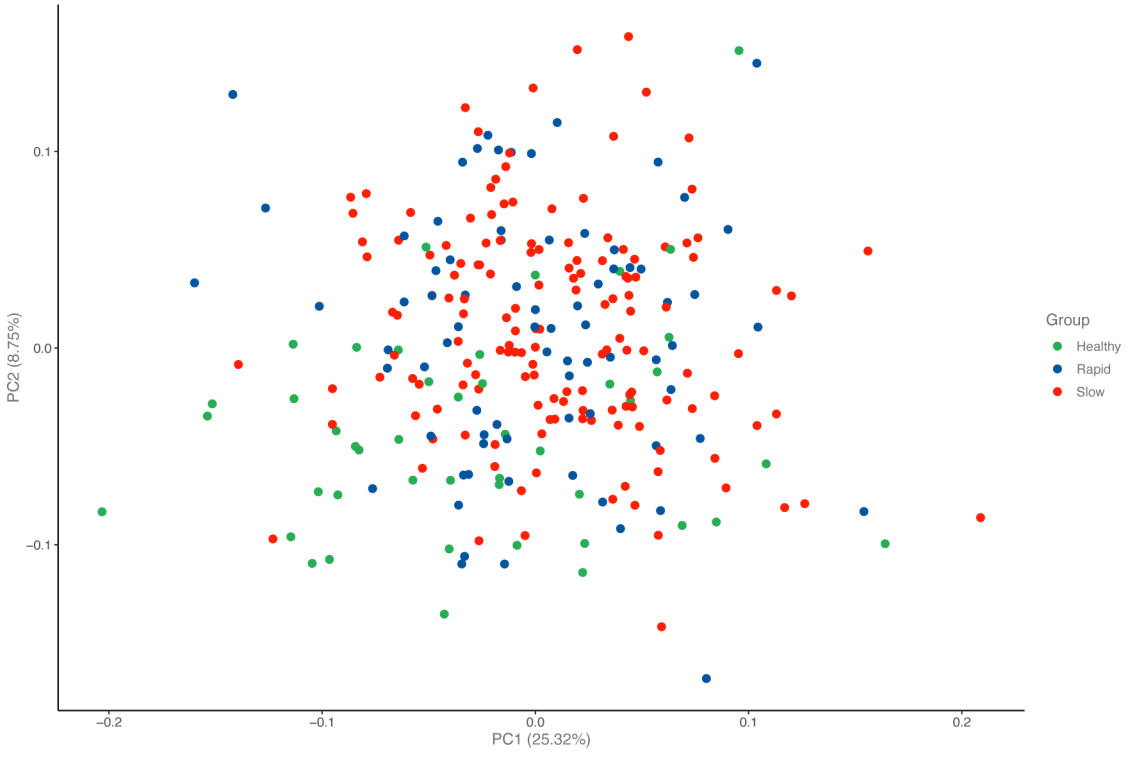

Figure 2 Principal component analysis samples in immune- oncology panel.

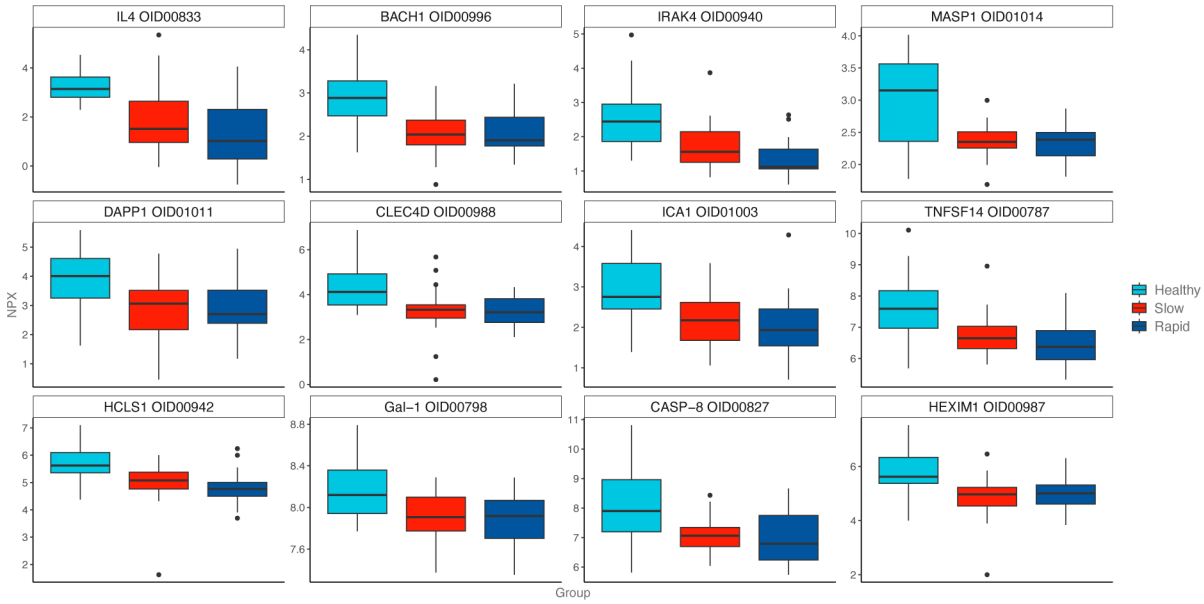

Figure 3 Boxplots of the 12 most significant biomarkers in ANOVA.

levels of three proteins in group *Rapid* compared with *Slow*, including carbonic anhydrase 9 (CAIX, p<0.001), corticosteroid 11-beta-dehydrogenase isozyme 1 (HSD11B1, p<0.01), and tumor necrosis factor receptor superfamily member 21 (TNFRSF21, p<0.05) (see online supplemental table 5).

Welch t-test: *T1D* cohort versus *Healthy* controls

Eighty-one biomarkers differed significantly between *T1D* (*Rapid* and *Slow* as one group) and *Healthy* at baseline (online supplemental table 4). Eleven of the 81 significant biomarkers were higher, and 70 were lower in the *T1D* cohort compared with *Healthy*. Cytokines with notable associations to the pathogenesis or prevention of type 1 diabetes included C-type lectin domain family 4 member D (CLEC4D, p<0.0001),[13] interleukin (IL)-4 (p<0.0001),[14] IL-12[15] (p<0.05), IL-13[16] (p<0.05), galectin-1 (Gal-1, p<0.0001),[17] tumor necrosis factor superfamily member 14 (TNFSF14, p<0.0001),[18] islet cell antigen 1 (ICA1, p<0.0001),[19] CD40 ligand[20] (p<0.05), mannan-binding lectin-associated serine proteases (MASP-1, p<0.0001),[21] peroxiredoxin-1 (PRDX1, p<0.01),[22] and latency-associated protein transforming growth factor beta 1 (LAP-TGF-beta 1, p<0.001).[23] (figure 4)

Linear mixed effects model (LMER)

Applying an LMER, the terms Time and Group (Rapid/Slow) were set as fixed and patient ID as random effects, respectively. Group term means that there is a difference between the groups independent of time, a significant Time term means that there is a difference over time independent of the group, and a significant interaction

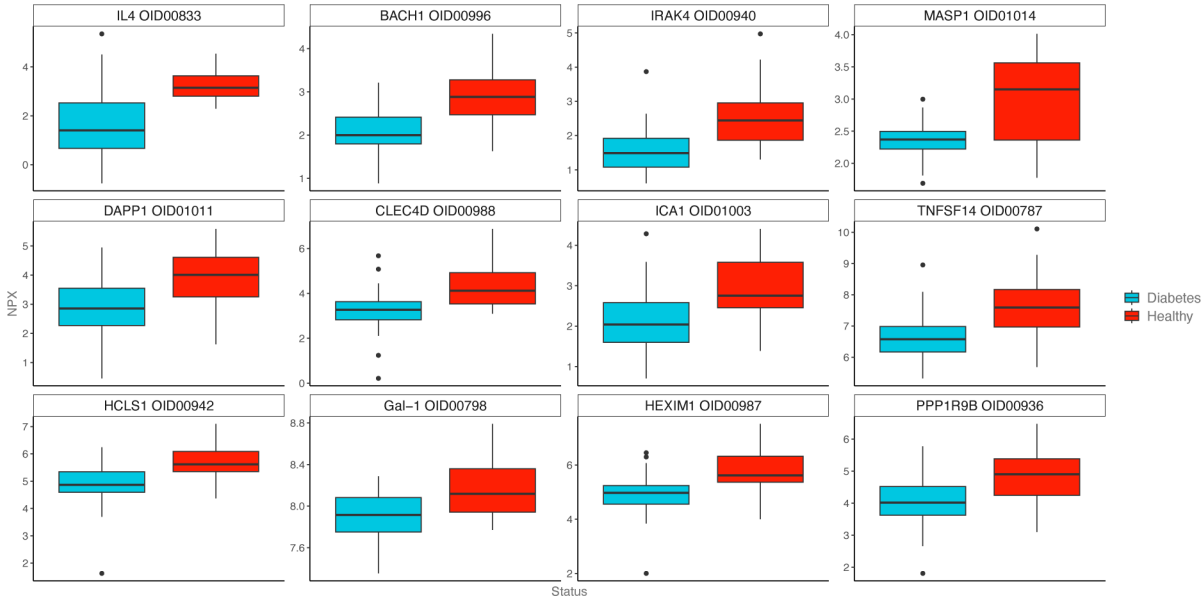

Figure 4 Boxplots of the 12 most significant biomarkers comparing groups *T1D* with *Healthy*. T1D, type 1 diabetes.

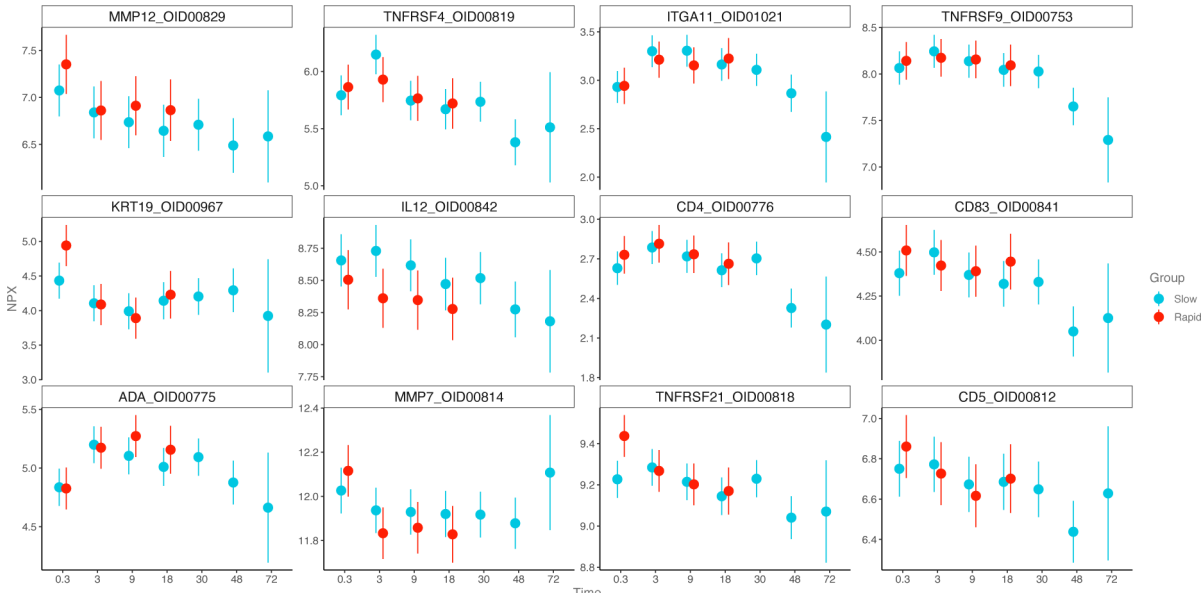

Figure 5 Point range plots of the top 12 most significant proteins for the Time term in linear mixed effects model.

(Time:Group) effect means that change over time depends on the group. Twenty-five proteins differed for the *Time* term (figure 5), including previously identified biomarkers of interest, IL-12 from debut and 3 to 18 months (both p<0.05), and Gal-1 from debut to 3 months (p<0.001) and from 3 to 18 months (p<0.05), with no difference for the *Group* term. One protein, the coxsackievirus B–adenovirus receptor (CAR), increased for the *Time:Group* interaction term, in the group *Rapid* from diagnosis to 9 (p<0.001) and 18 months (p<0.01), respectively. Integrin beta-6 (ITGB6) also increased in the *Time:Group* term for group *Rapid* but was not statistically significant after adjusting for multiple testing (p=0.07).

CONCLUSIONS/DISCUSSION

The rate of progression to complete beta cell failure in type 1 diabetes is heterogeneous. The variation is associated with clinical characteristics, but differences in the underlying biochemistry remain largely undefined. Identifying biomarkers associated with the disease progression rate could unveil underlying mechanisms and be of prognostic value, especially when selecting suitable intervention therapies. To investigate this, we analyzed 184 biomarkers by PEA in 46 children newly diagnosed with type 1 diabetes until loss of residual C-peptide secretion and compared baseline values with 45 healthy controls. As the study was performed retrospectively, we could classify participants with type 1 diabetes as progressing rapidly or slowly.

When comparing *T1D* with *Healthy*, 81 of the 184 analyzed proteins differed significantly. This notably includes several proteins associated with type 1 diabetes pathogenesis or protection and mainly confirms earlier findings. IL-4 was lower in the *T1D* cohort and has been associated with both protection against autoimmunity[14] as well as type 1 diabetes debut,[24 25] indeed, the IL-4 receptor is expressed in pancreatic islets.[26] IL-13, which is mostly known as a Th2 cytokine with anti-inflammatory properties,[27] was found to be higher in the *T1D* cohort, which was somewhat surprising considering that previous studies have found that the IL-13 production is decreased in patients with type 1 diabetes and individuals at risk of type 1 diabetes.[28 29] Also, experimental in vitro studies have found that IL-13 can reduce beta-cell apoptosis.[30] CLEC4D was lower in the *T1D* cohort, consistent with previous research associating low CLEC4D with proinflammatory states.[31] This corroborates recent findings linking significantly lower concentrations of CLEC4D with positive type 1 diabetes autoantibodies in human pancreata.[13] PRDX1[22] is an antioxidant with immunoregulatory properties that were lower in *T1D*. LAP-TGF-beta 1[23] is the upstream pro-protein of immunoregulator TGF-beta 1 and was lower in *T1D*. Notably, TGF-beta 1 dissociates from LAP and becomes active on interaction with primarily integrin ITGAV:ITGB6[32] and can promote Th17 or Treg differentiation in a concentration-dependent manner. High concentrations of TGF-beta 1 are shown to downregulate IL-17 expression in favor of Tregs, while low concentrations can promote Th17 differentiation.[33]

Soluble lectin Gal-1 was lower in the *T1D* cohort and is an anti-inflammatory cytokine[34] involved in several autoimmune diseases, including type 1 diabetes.[17] In addition, studies in non-obese diabetic mice associate Gal-1[35] and TNFSF14 (aka LIGHT)[18] with reversal of beta cell autoimmunity and insulitis, respectively.

Overall, lower levels of IL-4, Gal-1, TNFSF14, and higher levels of IL-12 in the *T1D* cohort are in line with a Th1-dominated immune response and previously published data. Higher IL-13 in the *T1D* cohort, a cytokine primarily associated with Th2 cells and immune tolerance, was unexpected and seemingly at odds with the Th1/Th2 paradigm of autoimmunity.

Our results primarily point to a lack of protective proteins in the *T1D* cohort compared with *Healthy*. In fact, cytokine levels were mostly lower in cohort *T1D*, possibly representing an inability to moderate an overzealous immune system attacking pancreatic beta cells. Incongruously, other biomarkers associated with type 1 diabetes pathogenesis, such as autoantibody ICA1,[19] immune cell activator CD40-ligand,[20] and complement activator MASP-1,[21] that can be raised in type 1 diabetes, were also lower in the *T1D* group, illustrating the complexity and heterogeneity of the type 1 diabetes pathogenesis.

The ANOVA showed higher levels of proteins CAIX, HSD11B1, and TNFRSF21 in group *Rapid* versus *Slow*. CAIX is involved in the L-Arginine/Nitric Oxide pathway and has previously been found to be altered in children with type 1 diabetes,[36] but it has not previously been associated with rapid disease progression per se. HSD11B1 is a catalyst for the reversible conversion of cortisone to cortisol and is expressed in various tissues. Nocturnal HSD11B1 has previously been found to be higher in children with type 1 diabetes[37] and has been found to increase insulin resistance.[38] HSD11B1 is also expressed in alpha and beta cells and is demonstrated to blunt glucose-stimulated insulin secretion.[39 40] Hence, a higher HSD11B1 could speed up beta cell exhaustion via increased peripheral insulin resistance and reduced insulin secretion. This may partly explain the lower stimulated C-peptide in the *Rapid* group, suggesting that there could be a functional impairment of beta cells in addition to direct destruction. TNFRSF21 is involved with the negative regulation of Th2-cell activation and cytokine release.[41] Upregulated TNFRSF21 and lower activation of the immune modulatory Th2-dependent pathway are in line with a more aggressive autoimmune attack in group *Rapid*.

In the linear mixed model, we found that none of the analyzed proteins differed in the *Group* term (*Rapid* vs *Slow*). However, 25 proteins, including ITGB6, IL-12, and Gal-1, differed in the *Time* term, suggesting an involvement in disease progression. Interestingly, ITGB6 has recently been found to be a target of autoantibodies in inflammatory bowel disease[42] suggesting that it could be involved in autoimmunity.

Interestingly, in the combined *Time:Group* interaction term only one marker, namely CAR, was altered significantly and found to increase in the *Rapid* group from debut to 9 and 18 months, respectively. ITGB6 was also altered over time depending on the group, but this was not significant after adjusting for multiple testing. It is, however, striking that CAR and ITGB6 are functionally similar, being trans-membrane, ligand-binding, signaling proteins involved in cell–cell interaction and autoimmunity. CARs are present on beta cells' surface and are a known port of entry for adenovirus and coxsackievirus, potentially triggering autoimmunity and insulitis.[43 44] ITGB6 is part of a dimer receptor together with integrin alpha 5 (ITGAV) that binds coxsackievirus and is expressed in pancreatic islet cells.[45] It is striking that this port of entry for viruses also activates TGF-beta 1, a known regulator of the T-helper 17 cell (Th17) pathway. Numerous epidemiological and clinical investigations support an association between enteroviruses, particularly coxsackievirus B, and autoimmune type 1 diabetes.[46 47] Although the mechanism remains incompletely understood, experimental findings are suggestive of molecular mimicry or bystander T-cell activation.[47] In this study, the change in CARs is specific to patients with rapidly progressing type 1 diabetes, which suggests an association between CARs and disease progression.

A strength of this study is the collection of initial blood samples shortly after type 1 diabetes diagnosis, unlike the wider definition of new-onset type 1 diabetes, which in some studies may extend up to a year after diagnosis. A limitation of the study is the inherent difficulty in interpreting how cytokine levels in peripheral blood mirror the immune response local to the pancreas. However, this approach does not compromise our aim to find prognostic biomarkers of type 1 diabetes progression rate. Furthermore, it is a limitation that 12 of 184 proteins (eg, IL-35, interferon, tumor necrosis factor) failed QC of the normalization between assays and were excluded from analyses. However, these biomarkers are deemed unlikely to separate the overlapping PCA plots. Also, the sample size of the study could limit the detection of more discrete differences between the groups.

In summary, the main aim of our study was to find biomarkers predictive of C-peptide decline in children with new-onset type 1 diabetes, but of the analyzed biomarkers, we found no clear separation in patterns that could separate the *Rapid*, *Slow*, and *Healthy* groups at baseline. However, we did find some markers which may be useful in predicting the decline of C-peptide. Also, these could potentially be important for our understanding of the type 1 diabetes pathogeneses, which merits further investigation.

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Contributors JL initiated and designed the study. JL coordinated and recruited the study subjects. JL, AG, and DE collected the data, and AG created the clinical descriptive database and analyzed the descriptive data. DE and PL created the PEA database. PL, DE, JL, and P-OC analyzed the data. PL drafted the first manuscript and DE edited the draft. All authors read and critically reviewed the manuscript and, in the final stage, approved the final version for publication. DE is guarantor.

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Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval The Research Ethics Committee of the Faculty of Health Sciences, Linköping University, approved the study with reference number 03-244. The ABIS study has ethical approval from the Research Ethics Committees of the Faculty of Health Science at Linköping University, Sweden, Ref. 1997/96287 and 2003/03-092 and the Medical Faculty of Lund University, Sweden (Dnr 99227, Dnr 99321). Participants gave informed consent to participate in the study before taking part.

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Data availability statement Data are available upon reasonable request.

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Protein assay list

Olink® Target 96 Immuno-Oncology

Product number: 95311

Adenosine deaminase (ADA)	P00813	C-X-C motif chemokine 11 (CXCL11)	O14625
Adhesion G-protein coupled receptor G1 (ADGRG1)	Q9Y653	C-X-C motif chemokine 13 (CXCL13)	O43927
Angiopoietin-1 (ANGPT1)	Q15389	C-X-C motif chemokine 5 (CXCL5)	P42830
Angiopoietin-1 receptor (TIE2)	Q02763	C-X-C motif chemokine 9 (CXCL9)	Q07325
Angiopoietin-2 (ANGPT2)	O15123	Cytotoxic and regulatory T-cell molecule (CRTAM)	O95727
Arginase-1 (ARG1)	P05089	Decorin (DCN)	P07585
Carbonic anhydrase 9 (CAIX)	Q16790	Fibroblast growth factor 2 (FGF2)	P09038
Caspase-8 (CASP-8)	Q14790	Fractalkine (CX3CL1)	P78423
C-C motif chemokine 13 (MCP-4)	Q99616	Galectin-1 (Gal-1)	P09382
C-C motif chemokine 17 (CCL17)	Q92583	Galectin-9 (Gal-9)	O00182
C-C motif chemokine 19 (CCL19)	Q99731	Granzyme A (GZMA)	P12544
C-C motif chemokine 2 (MCP-1)	P13500	Granzyme B (GZMB)	P10144
C-C motif chemokine 20 (CCL20)	P78556	Granzyme H (GZMH)	P20718
C-C motif chemokine 23 (CCL23)	P55773	Heme oxygenase 1 (HO-1)	P09601
C-C motif chemokine 3 (CCL3)	P10147	Hepatocyte growth factor (HGF)	P14210
C-C motif chemokine 4 (CCL4)	P13236	ICOS ligand (ICOSLG)	O75144
C-C motif chemokine 7 (MCP-3)	P80098	Interferon gamma (IFN-gamma)	P01579
C-C motif chemokine 8 (MCP-2)	P80075	Interleukin-1 alpha (IL-1 alpha)	P01583
CD27 antigen (CD27)	P26842	Interleukin-10 (IL10)	P22301
CD40 ligand (CD40-L)	P29965	Interleukin-12 (IL12)	P29459, P29460
CD40L receptor (CD40)	P25942	Interleukin-12 receptor subunit beta-1 (IL12RB1)	P42701
CD70 antigen (CD70)	P32970	Interleukin-13 (IL13)	P35225
CD83 antigen (CD83)	Q01151	Interleukin-15 (IL15)	P40933
C-X-C motif chemokine 1 (CXCL1)	P09341	Interleukin-18 (IL18)	Q14116
C-X-C motif chemokine 10 (CXCL10)	P02778	Interleukin-2 (IL2)	P60568

Table continues on reverse ▶

Interleukin-33 (IL33)	O95760	Pleiotrophin (PTN)	P21246
Interleukin-4 (IL4)	P05112	Pro-epidermal growth factor (EGF)	P01133
Interleukin-5 (IL5)	P05113	Programmed cell death 1 ligand 1 (PD-L1)	Q9NZQ7
Interleukin-6 (IL6)	P05231	Programmed cell death 1 ligand 2 (PD-L2)	Q9BQ51
Interleukin-7 (IL7)	P13232	Programmed cell death protein 1 (PDCD1)	Q15116
Interleukin-8 (IL8)	P10145	Stromal cell-derived factor 1 (CXCL12)	P48061
Killer cell immunoglobulin-like receptor 3DL1 (KIR3DL1)	P43629	T-cell surface glycoprotein CD4 (CD4)	P01730
Latency-associated peptide transforming growth factor beta-1 (LAP TGF-beta-1)	P01137	T-cell surface glycoprotein CD5 (CD5)	P06127
Lymphocyte activation gene 3 protein (LAG3)	P18627	T-cell surface glycoprotein CD8 alpha chain (CD8A)	P01732
Lysosome-associated membrane glycoprotein 3 (LAMP3)	Q9UQV4	T-cell-specific surface glycoprotein CD28 (CD28)	P10747
Macrophage colony-stimulating factor 1 (CSF-1)	P09603	TNF-related apoptosis-inducing ligand (TRAIL)	P50591
Macrophage metalloproteinase-12 (MMP12)	P39900	Tumor necrosis factor (TNF)	P01375
Matrix metalloproteinase-7 (MMP7)	P09237	Tumor necrosis factor ligand superfamily member 12 (TWEAK)	O43508
MHC class I polypeptide-related sequence A/B (MIC-A/B)	Q29983, Q29980	Tumor necrosis factor ligand superfamily member 14 (TNFSF14)	O43557
Mucin-16 (MUC-16)	Q8WXI7	Tumor necrosis factor ligand superfamily member 6 (FASLG)	P48023
Natural cytotoxicity triggering receptor (NCR1)	O76036	Tumor necrosis factor receptor superfamily member 12A (TNFRSF12A)	Q9NP84
Natural killer cell receptor 2B4 (CD244)	Q9BZW8	Tumor necrosis factor receptor superfamily member 21 (TNFRSF21)	O75509
Natural killer cells antigen CD94 (KLRD1)	Q13241	Tumor necrosis factor receptor superfamily member 4 (TNFRSF4)	P43489
Nitric oxide synthase, endothelial (NOS3)	P29474	Tumor necrosis factor receptor superfamily member 9 (TNFRSF9)	Q07011
Placenta growth factor (PGF)	P49763	Vascular endothelial growth factor A (VEGFA)	P15692
Platelet-derived growth factor subunit B (PDGF subunit B)	P01127	Vascular endothelial growth factor receptor 2 (VEGFR-2)	P35968

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Protein assay list

Olink® Target 96 Immune Response

Product number: 95320

Allergin-1 (MILR1)	Q7Z6M3	Eotaxin (CCL11)	P51671
Amphiregulin (AR) (AREG)	P15514	Eukaryotic translation initiation factor 4 gamma 1 (EIF4G1)	Q04637
Aryl hydrocarbon receptor nuclear translocator (ARNT)	P27540	Eukaryotic translation initiation factor 5A-1 (EIF5A)	P63241
Baculoviral IAP repeat-containing protein 2 (BIRC2)	Q13490	Fc receptor-like protein 3 (FCRL3)	Q96P31
Beta-galactosidase (GLB1)	P16278	Fc receptor-like protein 6 (FCRL6)	Q6DN72
Butyrophilin subfamily 3 member A2 (BTN3A2)	P78410	Fibroblast growth factor 2 (FGF2)	P09038
CD83 antigen (CD83)	Q01151	FXYD domain-containing ion transport regulator 5 (FXYD5)	Q96DB9
Contactin-associated protein-like 2 (CNTNAP2)	Q9UHC6	Hematopoietic lineage cell-specific protein (HCLS1)	P14317
Corneodesmosin (CDSN)	Q15517	Histamine N-methyltransferase (HNMT)	P50135
Corticosteroid 11-beta-dehydrogenase isozyme 1 (HSD11B1)	P28845	Importin subunit alpha-5 (KPNA1)	P52294
Coxsackievirus and adenovirus receptor (CXADR)	P78310	Inactive dipeptidyl peptidase 10 (DPP10)	Q8N608
C-type lectin domain family 4 member A (CLEC4A)	Q9UMR7	Integral membrane protein 2A (ITM2A)	O43736
C-type lectin domain family 4 member C (CLEC4C)	Q8WTT0	Integrin alpha-6 (ITGA6)	P23229
C-type lectin domain family 4 member D (CLEC4D)	Q8WXI8	Integrin alpha-11 (ITGA11)	Q9UKX5
C-type lectin domain family 4 member G (CLEC4G)	Q6UXB4	Integrin beta-6 (ITGB6)	P18564
C-type lectin domain family 6 member A (CLEC6A)	Q6EIG7	Interferon lambda receptor 1 (IFNLR1)	Q8IU57
C-type lectin domain family 7 member A (CLEC7A)	Q9BXN2	Interferon regulatory factor 9 (IRF9)	Q00978
Cytoskeleton-associated protein 4 (CKAP4)	Q07065	Interleukin-1 receptor-associated kinase 1 (IRAK1)	P51617
Diacylglycerol kinase zeta (DGKZ)	Q13574	Interleukin-1 receptor-associated kinase 4 (IRAK4)	Q9NWZ3
Discoidin, CUB and LCCL domain-containing protein 2 (DCBLD2)	Q96PD2	Interleukin-5 (IL5)	P05113
DNA fragmentation factor subunit alpha (DFFA)	O00273	Interleukin-6 (IL6)	P05231
Dual adapter for phosphotyrosine and 3-phosphotyrosine and 3-phosphoinositide (DAPP1)	Q9UN19	Interleukin-10 (IL10)	P22301
Dynactin subunit 1 (DCTN1)	Q14203	Interleukin-12 receptor subunit beta-1 (IL12RB1)	P42701
E3 ubiquitin-protein ligase TRIM21 (TRIM21)	P19474	Islet cell autoantigen 1 (ICA1)	Q05084
Egl nine homolog 1 (EGLN1)	Q9GZT9	Keratin, type I cytoskeletal 19 (KRT19)	P08727

Table continues on reverse ▶

Leukocyte immunoglobulin-like receptor subfamily B member 4 (LILRB4)	Q8NHJ6	Protein HEXIM1 (HEXIM1)	O94992
Lymphocyte activation gene 3 protein (LAG3)	P18627	Protein kinase C theta type (PRKCQ)	Q04759
Lymphocyte antigen 75 (LY75)	O60449	Protein sprouty homolog 2 (SPRY2)	O43597
Lysosome-associated membrane glycoprotein 3 (LAMP3)	Q9UQV4	Protein-arginine deiminase type-2 (PADI2)	Q9Y2J8
Mannan-binding lectin serine protease 1 (MASP1)	P48740	SH2 domain-containing protein 1A (SH2D1A)	O60880
Merlin (NF2)	P35240	SH2B adapter protein 3 (SH2B3)	Q9UQQ2
Methylated-DNA--protein-cysteine methyltransferase (MGMT)	P16455	Signaling threshold-regulating transmembrane adapter 1 (SIT1)	Q9Y3P8
Natural cytotoxicity triggering receptor 1 (NCR1)	O76036	SRSF protein kinase 2 (SRPK2)	P78362
Natural killer cells antigen CD94 (KLRD1)	Q13241	Stanniocalcin-1 (STC1)	P52823
Neurabin-2 (PPP1R9B)	Q96SB3	Stromal cell-derived factor 1 (CXCL12)	P48061
Neurotrophin-4 (NTF4)	P34130	T-cell-specific surface glycoprotein CD28 (CD28)	P10747
Nuclear factor of activated T-cells, cytoplasmic 3 (NFATC3)	Q12968	Thioredoxin-dependent peroxide reductase, mitochondrial (PRDX3)	P30048
Parathyroid hormone/parathyroid hormone-related peptide receptor (PTH1R)	Q03431	TNF receptor-associated factor 2 (TRAF2)	Q12933
PC4 and SFRS1-interacting protein (PSIP1)	O75475	TRAF family member-associated NF-kappa-B activator (TANK)	Q92844
Peroxiredoxin-1 (PRDX1)	Q06830	Transcription factor AP-1 (JUN)	P05412
Peroxiredoxin-5, mitochondrial (PRDX5)	P30044	Transcription regulator protein BACH1 (BACH1)	O14867
Phosphoinositide 3-kinase adapter protein 1 (PIK3AP1)	Q6ZUJ8	Triggering receptor expressed on myeloid cells 1 (TREM1)	Q9NP99
Plexin-A4 (PLXNA4)	Q9HCM2	Tripartite motif-containing protein 5 (TRIM5)	Q9C035
Polypeptide N-acetylgalactosaminyltransferase 3 (GALNT3)	Q14435	Tryptase alpha/beta-1 (TPSAB1)	Q15661
Probable ATP-dependent RNA helicase DDX58 (DDX58)	O95786	Tumor necrosis factor receptor superfamily member EDAR (EDAR)	Q9UNE0
Protein FAM3B (FAM3B)	P58499	Zinc finger and BTB domain-containing protein 16 (ZBTB16)	Q05516

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1051, v2.0, 2022-06-14

Analysis

Only proteins that were normalized were used in the following analysis. In Table 2, assays that were not included in the analysis are listed.

Table 2: The lists of unnormalized proteins were not included in the analysis.

Assay	OlinkID	Panel
IFN-beta	OID00774	Olink Immuno-Oncology
IFN-gamma	OID00825	Olink Immuno-Oncology
IFN-gamma	OID05552	Olink Immuno-Oncology
IL-21	OID00834	Olink Immuno-Oncology
IL-35	OID00797	Olink Immuno-Oncology
IL15	OID05551	Olink Immuno-Oncology
KIR3DL1	OID05550	Olink Immuno-Oncology
LAG3	OID05553	Olink Immuno-Oncology
MUC-16	OID05549	Olink Immuno-Oncology
TNF	OID00838	Olink Immuno-Oncology
TNF	OID05554	Olink Immuno-Oncology
VEGFC	OID00784	Olink Immuno-Oncology

Welch two-sided T-test

Assay	Diabetes	Healthy	estimate	conf.low	conf.high	p.value	Adj.p.value	Threshold
IL4	1,65	3,25	-1,59	-2,05	-1,13	3,44E-09	4,69E-07	Sign
BACH1	2,07	2,85	-0,78	-1,02	-0,54	5,27E-09	4,69E-07	Sign
IRAK4	1,57	2,54	-0,97	-1,28	-0,66	1,60E-08	9,29E-07	Sign
MASP1	2,36	3,04	-0,68	-0,89	-0,47	2,09E-08	9,29E-07	Sign
DAPP1	2,86	3,95	-1,09	-1,47	-0,70	2,09E-07	7,46E-06	Sign
CLEC4D	3,25	4,33	-1,08	-1,47	-0,69	3,20E-07	9,12E-06	Sign
ICA1	2,09	2,94	-0,85	-1,16	-0,55	3,59E-07	9,12E-06	Sign
TNFSF14	6,66	7,60	-0,93	-1,29	-0,58	1,04E-06	2,31E-05	Sign
HCLS1	4,91	5,65	-0,74	-1,03	-0,46	1,46E-06	2,89E-05	Sign
Gal-1	7,90	8,16	-0,26	-0,36	-0,15	3,51E-06	6,25E-05	Sign
HEXIM1	4,91	5,69	-0,78	-1,10	-0,46	5,50E-06	8,89E-05	Sign
CASP-8	7,05	8,11	-1,06	-1,49	-0,62	6,93E-06	9,48E-05	Sign
PPP1R9B	4,00	4,83	-0,83	-1,18	-0,49	6,58E-06	9,48E-05	Sign
PIK3AP1	4,32	5,08	-0,76	-1,08	-0,44	9,49E-06	1,21E-04	Sign
TRIM21	3,49	4,35	-0,86	-1,22	-0,49	1,03E-05	1,22E-04	Sign
IL8	8,24	10,50	-2,26	-3,25	-1,27	1,79E-05	1,87E-04	Sign
PRDX5	7,37	7,99	-0,62	-0,89	-0,35	1,75E-05	1,87E-04	Sign
DCN	5,73	5,92	-0,19	-0,28	-0,10	3,84E-05	3,79E-04	Sign
GZMH	5,68	6,68	-1,00	-1,46	-0,54	4,60E-05	4,31E-04	Sign
CLEC6A	2,88	3,39	-0,50	-0,74	-0,27	4,94E-05	4,39E-04	Sign
LAP TGF-beta-1	3,21	3,72	-0,51	-0,74	-0,27	6,25E-05	5,30E-04	Sign
CCL23	11,49	11,88	-0,39	-0,58	-0,20	9,19E-05	7,43E-04	Sign
IRAK1	2,79	3,45	-0,66	-0,99	-0,33	1,70E-04	1,23E-03	Sign
GLB1	2,72	3,20	-0,48	-0,72	-0,24	1,68E-04	1,23E-03	Sign
ANG-1	10,82	11,23	-0,42	-0,63	-0,21	1,72E-04	1,23E-03	Sign
DFFA	6,04	6,68	-0,64	-0,97	-0,32	1,83E-04	1,25E-03	Sign
EGLN1	1,64	2,08	-0,45	-0,67	-0,22	2,01E-04	1,32E-03	Sign
PRDX1	2,59	3,20	-0,61	-0,92	-0,30	2,28E-04	1,45E-03	Sign
SIT1	3,25	3,89	-0,64	-0,97	-0,31	2,44E-04	1,50E-03	Sign
HGF	8,45	8,78	-0,33	-0,51	-0,16	3,28E-04	1,88E-03	Sign
CXCL12	1,46	1,64	-0,19	-0,29	-0,09	3,22E-04	1,88E-03	Sign
TREM1	-0,40	0,06	-0,46	-0,71	-0,21	3,45E-04	1,92E-03	Sign
PSIP1	2,23	2,99	-0,75	-1,16	-0,35	3,73E-04	1,95E-03	Sign
SRPK2	1,05	1,58	-0,53	-0,82	-0,25	3,69E-04	1,95E-03	Sign
CLEC4A	4,76	5,13	-0,38	-0,58	-0,17	4,88E-04	2,48E-03	Sign
SPRY2	2,63	3,20	-0,57	-0,88	-0,25	5,58E-04	2,76E-03	Sign
ADA	4,83	5,16	-0,33	-0,51	-0,15	6,02E-04	2,90E-03	Sign
TRIM5	4,12	4,83	-0,71	-1,11	-0,31	6,58E-04	3,08E-03	Sign
ARG1	3,93	4,63	-0,70	-1,10	-0,31	6,81E-04	3,11E-03	Sign
SH2D1A	2,67	3,34	-0,67	-1,04	-0,29	7,17E-04	3,19E-03	Sign
EDAR	3,67	4,39	-0,71	-1,12	-0,31	7,97E-04	3,46E-03	Sign
HSD11B1	3,14	3,43	-0,30	-0,46	-0,13	8,40E-04	3,56E-03	Sign

ITGA6	1,47	1,83	-0,36	-0,57	-0,15	8,95E-04	3,70E-03	Sign
TWEAK	10,43	10,73	-0,30	-0,47	-0,13	9,78E-04	3,96E-03	Sign
IRF9	2,85	3,35	-0,50	-0,79	-0,20	1,34E-03	5,32E-03	Sign
CD4	2,67	2,96	-0,28	-0,46	-0,11	1,52E-03	5,90E-03	Sign
EIF4G1	6,56	7,15	-0,58	-0,95	-0,22	1,90E-03	7,18E-03	Sign
BTN3A2	3,87	4,21	-0,34	-0,56	-0,13	2,01E-03	7,45E-03	Sign
PRKCQ	3,75	4,49	-0,74	-1,20	-0,28	2,05E-03	7,46E-03	Sign
DCTN1	5,17	5,74	-0,57	-0,93	-0,21	2,50E-03	8,90E-03	Sign
BIRC2	0,68	1,03	-0,35	-0,57	-0,12	2,76E-03	9,64E-03	Sign
FXYD5	1,47	1,87	-0,40	-0,66	-0,14	3,24E-03	1,11E-02	Sign
CXCL13	10,30	10,68	-0,38	-0,63	-0,13	3,79E-03	1,27E-02	Sign
CKAP4	5,39	5,72	-0,33	-0,55	-0,11	3,98E-03	1,31E-02	Sign
ITM2A	4,20	3,72	0,48	0,16	0,80	4,10E-03	1,33E-02	Sign
DDX58	3,35	4,05	-0,70	-1,18	-0,23	4,37E-03	1,39E-02	Sign
MMP12	7,18	6,77	0,41	0,13	0,69	4,47E-03	1,40E-02	Sign
CD40-L	8,97	9,57	-0,59	-1,01	-0,18	5,40E-03	1,60E-02	Sign
VEGFA	9,37	9,64	-0,27	-0,45	-0,08	5,26E-03	1,60E-02	Sign
KRT19	4,65	4,20	0,45	0,14	0,77	5,35E-03	1,60E-02	Sign
CLEC4G	3,34	3,64	-0,30	-0,51	-0,09	5,69E-03	1,66E-02	Sign
ICOSLG	6,72	6,94	-0,22	-0,37	-0,06	6,21E-03	1,75E-02	Sign
IL13	1,25	0,70	0,55	0,16	0,94	6,16E-03	1,75E-02	Sign
GZMB	4,44	4,93	-0,49	-0,84	-0,14	6,37E-03	1,77E-02	Sign
ZBTB16	3,97	4,55	-0,57	-0,99	-0,16	7,39E-03	1,99E-02	Sign
TANK	2,58	2,95	-0,37	-0,64	-0,10	7,49E-03	1,99E-02	Sign
IL12	8,59	8,27	0,31	0,09	0,54	7,39E-03	1,99E-02	Sign
IL7	5,72	6,10	-0,38	-0,67	-0,10	8,07E-03	2,10E-02	Sign
FGF2	0,58	0,77	-0,20	-0,34	-0,05	8,19E-03	2,10E-02	Sign
TNFRSF21	9,32	9,18	0,15	0,04	0,25	8,36E-03	2,10E-02	Sign
IL12RB1	3,03	2,79	0,24	0,06	0,42	8,36E-03	2,10E-02	Sign
EGF	11,34	11,75	-0,42	-0,73	-0,11	9,39E-03	2,32E-02	Sign
PDGF subunit B	12,63	12,77	-0,13	-0,24	-0,03	1,07E-02	2,61E-02	Sign
CCL19	11,27	10,99	0,28	0,07	0,49	1,11E-02	2,66E-02	Sign
CD83	4,43	4,22	0,21	0,05	0,38	1,16E-02	2,75E-02	Sign
CCL20	8,72	8,28	0,44	0,09	0,78	1,49E-02	3,50E-02	Sign
NCR1	3,09	3,35	-0,26	-0,47	-0,05	1,61E-02	3,72E-02	Sign
ADGRG1	1,67	1,84	-0,16	-0,30	-0,03	1,69E-02	3,85E-02	Sign
IL10	4,59	4,30	0,29	0,05	0,53	1,77E-02	3,99E-02	Sign
NF2	-1,20	-0,76	-0,43	-0,79	-0,07	2,01E-02	4,47E-02	Sign
AREG	3,00	3,43	-0,42	-0,78	-0,07	2,05E-02	4,51E-02	Sign
CCL4	9,25	9,83	-0,58	-1,09	-0,07	2,70E-02	5,86E-02	NS
ANGPT2	6,67	6,89	-0,22	-0,42	-0,02	2,91E-02	6,19E-02	NS
MGMT	4,91	5,31	-0,40	-0,75	-0,04	2,94E-02	6,19E-02	NS
CCL3	7,74	8,55	-0,81	-1,54	-0,08	2,96E-02	6,19E-02	NS
VEGFR-2	8,24	8,37	-0,12	-0,24	-0,01	3,12E-02	6,43E-02	NS
IL33	1,27	1,37	-0,10	-0,19	-0,01	3,14E-02	6,43E-02	NS
CDSN	3,34	3,55	-0,22	-0,42	-0,02	3,18E-02	6,44E-02	NS

TIE2	9,16	9,29	-0,13	-0,25	-0,01	3,57E-02	7,14E-02	NS
LY75	3,36	3,55	-0,19	-0,37	-0,01	3,64E-02	7,21E-02	NS
LAMP3	4,48	4,73	-0,25	-0,49	-0,01	4,12E-02	8,06E-02	NS
HO-1	13,03	13,23	-0,19	-0,38	0,00	4,48E-02	8,66E-02	NS
CXCL5	13,27	13,61	-0,35	-0,70	0,00	4,85E-02	9,28E-02	NS
CLEC7A	3,62	3,87	-0,25	-0,50	0,00	5,03E-02	9,53E-02	NS
IFNLR1	1,12	0,87	0,25	0,00	0,50	5,10E-02	9,56E-02	NS
IL6	3,83	4,44	-0,61	-1,23	0,01	5,55E-02	1,03E-01	NS
GZMA	6,83	7,01	-0,18	-0,36	0,01	5,78E-02	1,05E-01	NS
LILRB4	3,55	3,75	-0,19	-0,39	0,01	5,80E-02	1,05E-01	NS
IL6	2,83	3,42	-0,59	-1,21	0,03	6,21E-02	1,12E-01	NS
SH2B3	4,25	4,60	-0,34	-0,72	0,03	6,95E-02	1,24E-01	NS
FCRL3	2,27	2,47	-0,19	-0,41	0,02	7,34E-02	1,29E-01	NS
PLXNA4	8,05	8,44	-0,39	-0,85	0,07	9,67E-02	1,69E-01	NS
CCL17	10,41	10,68	-0,28	-0,61	0,06	1,06E-01	1,84E-01	NS
PTN	0,73	0,95	-0,23	-0,51	0,06	1,15E-01	1,96E-01	NS
CD8A	11,35	11,17	0,18	-0,04	0,40	1,15E-01	1,96E-01	NS
CXCL1	11,23	11,46	-0,23	-0,51	0,06	1,20E-01	2,02E-01	NS
HNMT	9,48	9,70	-0,22	-0,50	0,06	1,29E-01	2,14E-01	NS
CD5	6,80	6,97	-0,17	-0,39	0,05	1,34E-01	2,19E-01	NS
MCP-1	12,28	12,16	0,12	-0,04	0,28	1,34E-01	2,19E-01	NS
IL-1 alpha	-0,83	-1,05	0,22	-0,07	0,51	1,36E-01	2,21E-01	NS
TNFRSF12A	6,94	7,06	-0,12	-0,29	0,05	1,50E-01	2,39E-01	NS
MIC-A/B	4,87	5,44	-0,57	-1,36	0,21	1,50E-01	2,39E-01	NS
FAM3B	5,06	5,23	-0,17	-0,41	0,07	1,54E-01	2,43E-01	NS
TRAF2	3,96	4,22	-0,26	-0,63	0,11	1,63E-01	2,54E-01	NS
FCRL6	4,19	4,39	-0,20	-0,49	0,09	1,77E-01	2,75E-01	NS
CXCL12	2,35	2,43	-0,08	-0,20	0,04	1,80E-01	2,76E-01	NS
PDCD1	5,41	5,25	0,17	-0,08	0,41	1,88E-01	2,86E-01	NS
MCP-4	12,06	11,88	0,18	-0,10	0,47	2,05E-01	3,09E-01	NS
FGF2	1,21	1,33	-0,12	-0,31	0,07	2,11E-01	3,15E-01	NS
IL10	4,53	4,35	0,19	-0,11	0,48	2,16E-01	3,20E-01	NS
CD27	9,37	9,29	0,09	-0,05	0,23	2,18E-01	3,20E-01	NS
CD70	4,34	4,23	0,11	-0,07	0,28	2,20E-01	3,20E-01	NS
CLEC4C	5,15	5,30	-0,15	-0,40	0,09	2,22E-01	3,21E-01	NS
IL2	0,41	0,53	-0,12	-0,31	0,07	2,26E-01	3,24E-01	NS
KLRD1	7,21	7,38	-0,16	-0,44	0,11	2,37E-01	3,37E-01	NS
PRDX3	1,43	1,63	-0,20	-0,54	0,14	2,47E-01	3,49E-01	NS
IL12RB1	2,52	2,42	0,10	-0,07	0,28	2,52E-01	3,54E-01	NS
CD244	7,98	7,88	0,09	-0,08	0,26	2,85E-01	3,96E-01	NS
ITGB6	3,50	3,60	-0,10	-0,28	0,09	2,95E-01	4,07E-01	NS
NOS3	1,67	1,56	0,11	-0,10	0,32	3,14E-01	4,30E-01	NS
TNFRSF4	5,82	5,71	0,11	-0,10	0,32	3,16E-01	4,30E-01	NS
CCL11	8,79	8,87	-0,08	-0,25	0,09	3,32E-01	4,47E-01	NS
MILR1	4,66	4,77	-0,11	-0,35	0,12	3,44E-01	4,60E-01	NS
Gal-9	9,20	9,14	0,06	-0,07	0,19	3,52E-01	4,63E-01	NS

CNTNAP2	2,47	2,56	-0,09	-0,27	0,10	3,53E-01	4,63E-01	NS
TNFRSF9	8,09	8,00	0,09	-0,11	0,29	3,54E-01	4,63E-01	NS
GALNT3	2,28	2,39	-0,10	-0,32	0,12	3,59E-01	4,66E-01	NS
NCR1	4,63	4,55	0,08	-0,10	0,26	3,70E-01	4,77E-01	NS
IL18	10,47	10,36	0,11	-0,13	0,34	3,75E-01	4,80E-01	NS
CD83	4,25	4,16	0,09	-0,12	0,29	4,03E-01	5,09E-01	NS
PD-L1	6,42	6,35	0,08	-0,10	0,26	4,03E-01	5,09E-01	NS
MCP-2	8,98	9,11	-0,13	-0,45	0,19	4,14E-01	5,19E-01	NS
PADI2	1,56	1,73	-0,17	-0,60	0,25	4,18E-01	5,20E-01	NS
PGF	9,30	9,35	-0,05	-0,20	0,09	4,62E-01	5,69E-01	NS
LAG3	2,81	2,87	-0,06	-0,21	0,10	4,63E-01	5,69E-01	NS
IL5	3,48	3,72	-0,25	-0,97	0,48	5,04E-01	6,14E-01	NS
CXCL10	9,31	9,42	-0,11	-0,44	0,22	5,11E-01	6,19E-01	NS
TPSAB1	4,38	4,28	0,11	-0,23	0,45	5,29E-01	6,36E-01	NS
CD40	13,50	13,57	-0,07	-0,29	0,15	5,40E-01	6,46E-01	NS
KLRD1	7,24	7,17	0,07	-0,15	0,28	5,49E-01	6,48E-01	NS
KPNA1	1,47	1,27	0,20	-0,46	0,86	5,50E-01	6,48E-01	NS
ARNT	1,57	1,74	-0,17	-0,74	0,40	5,54E-01	6,48E-01	NS
JUN	1,40	1,24	0,17	-0,40	0,73	5,57E-01	6,48E-01	NS
DCBLD2	9,10	9,03	0,07	-0,17	0,31	5,70E-01	6,55E-01	NS
CXADR	2,08	2,14	-0,05	-0,23	0,13	5,72E-01	6,55E-01	NS
NFATC3	1,22	1,16	0,07	-0,17	0,31	5,74E-01	6,55E-01	NS
DGKZ	0,32	0,38	-0,05	-0,24	0,14	5,83E-01	6,56E-01	NS
NTF4	2,16	2,09	0,06	-0,16	0,29	5,84E-01	6,56E-01	NS
DPP10	2,26	2,32	-0,06	-0,28	0,16	5,86E-01	6,56E-01	NS
CD28	1,80	1,86	-0,05	-0,27	0,16	6,18E-01	6,88E-01	NS
ITGA11	2,94	2,99	-0,05	-0,25	0,15	6,29E-01	6,95E-01	NS
PTH1R	2,84	2,90	-0,06	-0,31	0,19	6,33E-01	6,95E-01	NS
STC1	6,53	6,47	0,06	-0,20	0,32	6,43E-01	6,99E-01	NS
TRAIL	9,12	9,08	0,04	-0,12	0,19	6,47E-01	6,99E-01	NS
CSF-1	9,44	9,47	-0,02	-0,13	0,08	6,48E-01	6,99E-01	NS
MMP7	12,07	12,10	-0,02	-0,15	0,11	7,28E-01	7,80E-01	NS
FASLG	8,04	8,07	-0,03	-0,20	0,15	7,44E-01	7,93E-01	NS
CXCL11	11,87	11,91	-0,03	-0,27	0,20	7,77E-01	8,23E-01	NS
IL5	2,16	2,23	-0,07	-0,71	0,56	8,17E-01	8,61E-01	NS
LAMP3	5,80	5,77	0,02	-0,19	0,24	8,26E-01	8,65E-01	NS
CRTAM	6,07	6,09	-0,02	-0,24	0,20	8,47E-01	8,80E-01	NS
EIF5A	1,68	1,71	-0,03	-0,36	0,30	8,50E-01	8,80E-01	NS
CD28	3,32	3,33	-0,01	-0,21	0,18	8,82E-01	9,07E-01	NS
CAIX	5,22	5,21	0,01	-0,16	0,19	8,94E-01	9,14E-01	NS
CX3CL1	6,97	6,96	0,01	-0,15	0,17	9,16E-01	9,32E-01	NS
PD-L2	3,75	3,74	0,01	-0,16	0,17	9,39E-01	9,50E-01	NS
CXCL9	8,47	8,48	-0,01	-0,30	0,29	9,65E-01	9,66E-01	NS
MCP-3	4,30	4,31	-0,01	-0,34	0,32	9,66E-01	9,66E-01	NS

Table 5. ANOVA Rapid, Slow and Healthy adjusted for multiplicity.

Assay	statistic	p.value	Adj.P-value	Threshold
IL4	24,65	4,14E-09	7,38E-07	Sign
BACH1	21,16	3,14E-08	1,40E-06	Sign
IRAK4	21,24	2,96E-08	1,40E-06	Sign
MASP1	21,25	2,96E-08	1,40E-06	Sign
DAPP1	15,69	1,49E-06	5,30E-05	Sign
CLEC4D	15,19	2,16E-06	5,49E-05	Sign
ICA1	15,23	2,10E-06	5,49E-05	Sign
TNFSF14	14,75	3,05E-06	6,79E-05	Sign
HCLS1	13,47	7,90E-06	1,56E-04	Sign
Gal-1	12,14	2,24E-05	3,98E-04	Sign
CASP-8	11,83	2,85E-05	4,45E-04	Sign
HEXIM1	11,75	3,00E-05	4,45E-04	Sign
PPP1R9B	11,48	3,71E-05	5,08E-04	Sign
IL8	11,04	5,34E-05	6,18E-04	Sign
PIK3AP1	11,07	5,13E-05	6,18E-04	Sign
TRIM21	10,97	5,56E-05	6,18E-04	Sign
PRDX5	10,32	9,42E-05	9,86E-04	Sign
HSD11B1	9,88	1,34E-04	1,33E-03	Sign
CXCL12	9,65	1,63E-04	1,53E-03	Sign
GZMH	9,52	1,82E-04	1,62E-03	Sign
DCN	9,37	2,07E-04	1,67E-03	Sign
LAP TGF-beta-1	9,39	2,03E-04	1,67E-03	Sign
CLEC6A	9,00	2,77E-04	2,14E-03	Sign
CCL23	8,81	3,28E-04	2,43E-03	Sign
PSIP1	8,36	4,75E-04	3,38E-03	Sign
GLB1	8,16	5,60E-04	3,83E-03	Sign
DFFA	7,89	7,03E-04	4,47E-03	Sign
TNFRSF21	7,93	6,84E-04	4,47E-03	Sign
EGLN1	7,73	8,09E-04	4,64E-03	Sign
IRAK1	7,79	7,66E-04	4,64E-03	Sign
PRDX1	7,75	7,92E-04	4,64E-03	Sign
ANG-1	7,70	8,36E-04	4,65E-03	Sign
SIT1	7,41	1,06E-03	5,70E-03	Sign
HGF	7,26	1,22E-03	6,37E-03	Sign
TREM1	7,00	1,51E-03	7,69E-03	Sign
SRPK2	6,95	1,58E-03	7,81E-03	Sign
CAIX	6,89	1,67E-03	7,82E-03	Sign
KRT19	6,91	1,64E-03	7,82E-03	Sign
SPRY2	6,64	2,06E-03	9,41E-03	Sign
CLEC4A	6,54	2,25E-03	1,00E-02	Sign
EDAR	6,43	2,48E-03	1,05E-02	Sign
SH2D1A	6,44	2,45E-03	1,05E-02	Sign
ARG1	6,32	2,73E-03	1,13E-02	Sign

ADA	6,27	2,87E-03	1,16E-02	Sign
TRIM5	6,24	2,93E-03	1,16E-02	Sign
TWEAK	6,06	3,42E-03	1,32E-02	Sign
ITGA6	5,84	4,15E-03	1,57E-02	Sign
CD4	5,73	4,61E-03	1,71E-02	Sign
IRF9	5,66	4,88E-03	1,77E-02	Sign
MMP12	5,56	5,34E-03	1,90E-02	Sign
EIF4G1	5,14	7,72E-03	2,69E-02	Sign
BTN3A2	5,04	8,44E-03	2,89E-02	Sign
PRKCQ	5,02	8,65E-03	2,90E-02	Sign
BIRC2	4,84	1,02E-02	3,36E-02	Sign
DCTN1	4,79	1,06E-02	3,42E-02	Sign
ITM2A	4,65	1,21E-02	3,84E-02	Sign
NCR1	4,61	1,24E-02	3,88E-02	Sign
FXYD5	4,59	1,27E-02	3,89E-02	Sign
CXCL13	4,51	1,37E-02	4,15E-02	Sign
ICOSLG	4,41	1,50E-02	4,45E-02	Sign
CKAP4	4,36	1,56E-02	4,55E-02	Sign
GZMB	4,27	1,70E-02	4,89E-02	Sign
DDX58	4,23	1,76E-02	4,93E-02	Sign
VEGFA	4,23	1,77E-02	4,93E-02	Sign
IL12	4,15	1,90E-02	5,12E-02	NS
MIC-A/B	4,16	1,87E-02	5,12E-02	NS
CCL4	4,07	2,04E-02	5,36E-02	NS
CD40-L	4,07	2,05E-02	5,36E-02	NS
CLEC4G	4,03	2,11E-02	5,44E-02	NS
CD83	3,99	2,20E-02	5,61E-02	NS
IL13	3,90	2,38E-02	5,97E-02	NS
EGF	3,87	2,46E-02	6,08E-02	NS
FGF2	3,84	2,52E-02	6,16E-02	NS
IL7	3,75	2,74E-02	6,24E-02	NS
LY75	3,76	2,70E-02	6,24E-02	NS
PDGF subunit B	3,77	2,69E-02	6,24E-02	NS
TANK	3,76	2,71E-02	6,24E-02	NS
ZBTB16	3,78	2,66E-02	6,24E-02	NS
IL12RB1	3,73	2,80E-02	6,31E-02	NS
IL-1 alpha	3,70	2,86E-02	6,36E-02	NS
CCL3	3,64	3,04E-02	6,69E-02	NS
KPNA1	3,53	3,34E-02	7,24E-02	NS
CCL19	3,36	3,94E-02	8,45E-02	NS
IL10	3,25	4,33E-02	9,18E-02	NS
CX3CL1	3,17	4,68E-02	9,80E-02	NS
TIE2	3,16	4,73E-02	9,80E-02	NS
CCL20	3,12	4,94E-02	1,01E-01	NS
DCBLD2	3,08	5,08E-02	1,03E-01	NS
ADGRG1	2,97	5,65E-02	1,13E-01	NS

NF2	2,94	5,82E-02	1,15E-01	NS
ITGB6	2,90	6,00E-02	1,17E-01	NS
CLEC7A	2,87	6,20E-02	1,20E-01	NS
AREG	2,85	6,29E-02	1,20E-01	NS
ANGPT2	2,77	6,79E-02	1,29E-01	NS
VEGFR-2	2,64	7,74E-02	1,45E-01	NS
NCR1	2,60	8,03E-02	1,49E-01	NS
IL6	2,58	8,18E-02	1,50E-01	NS
HO-1	2,54	8,43E-02	1,53E-01	NS
CDSN	2,53	8,54E-02	1,54E-01	NS
LAMP3	2,49	8,88E-02	1,58E-01	NS
MGMT	2,41	9,53E-02	1,68E-01	NS
GALNT3	2,36	1,01E-01	1,74E-01	NS
IL33	2,37	9,99E-02	1,74E-01	NS
IL6	2,33	1,04E-01	1,77E-01	NS
CCL11	2,30	1,06E-01	1,80E-01	NS
HNMT	2,11	1,27E-01	2,12E-01	NS
LILRB4	2,11	1,27E-01	2,12E-01	NS
CXCL5	2,07	1,33E-01	2,17E-01	NS
FGF2	2,07	1,32E-01	2,17E-01	NS
EIF5A	2,06	1,34E-01	2,17E-01	NS
FAM3B	1,99	1,43E-01	2,29E-01	NS
IFNLR1	1,95	1,48E-01	2,35E-01	NS
SH2B3	1,92	1,53E-01	2,40E-01	NS
GZMA	1,90	1,55E-01	2,42E-01	NS
FCRL3	1,76	1,79E-01	2,77E-01	NS
CXCL1	1,71	1,88E-01	2,88E-01	NS
ARNT	1,62	2,03E-01	3,09E-01	NS
PLXNA4	1,60	2,08E-01	3,14E-01	NS
PDCD1	1,59	2,10E-01	3,14E-01	NS
FASLG	1,54	2,21E-01	3,26E-01	NS
IL18	1,53	2,21E-01	3,26E-01	NS
CD5	1,40	2,51E-01	3,66E-01	NS
CCL17	1,32	2,72E-01	3,94E-01	NS
PTN	1,31	2,76E-01	3,96E-01	NS
CD8A	1,27	2,85E-01	4,06E-01	NS
CNTNAP2	1,21	3,03E-01	4,28E-01	NS
CXCL12	1,18	3,11E-01	4,33E-01	NS
MCP-4	1,18	3,11E-01	4,33E-01	NS
PD-L1	1,15	3,21E-01	4,43E-01	NS
MCP-1	1,13	3,27E-01	4,48E-01	NS
IL10	1,12	3,31E-01	4,50E-01	NS
LAG3	1,09	3,42E-01	4,61E-01	NS
MILR1	1,04	3,58E-01	4,75E-01	NS
TNFRSF12A	1,05	3,55E-01	4,75E-01	NS
TRAF2	1,02	3,64E-01	4,79E-01	NS

CD27	1,01	3,68E-01	4,82E-01	NS
FCRL6	0,93	3,98E-01	5,17E-01	NS
IL2	0,89	4,16E-01	5,29E-01	NS
KLRD1	0,89	4,16E-01	5,29E-01	NS
TPSAB1	0,90	4,11E-01	5,29E-01	NS
NFATC3	0,83	4,39E-01	5,54E-01	NS
CSF-1	0,82	4,46E-01	5,59E-01	NS
CD83	0,78	4,60E-01	5,73E-01	NS
CLEC4C	0,76	4,69E-01	5,75E-01	NS
PRDX3	0,77	4,68E-01	5,75E-01	NS
CD70	0,76	4,73E-01	5,76E-01	NS
STC1	0,68	5,10E-01	6,18E-01	NS
IL12RB1	0,66	5,21E-01	6,23E-01	NS
LAMP3	0,66	5,21E-01	6,23E-01	NS
TNFRSF4	0,65	5,25E-01	6,23E-01	NS
TNFRSF9	0,61	5,46E-01	6,43E-01	NS
PD-L2	0,60	5,51E-01	6,45E-01	NS
CD244	0,58	5,65E-01	6,49E-01	NS
NOS3	0,58	5,62E-01	6,49E-01	NS
PGF	0,58	5,63E-01	6,49E-01	NS
CXCL11	0,52	5,99E-01	6,84E-01	NS
JUN	0,48	6,18E-01	7,01E-01	NS
Gal-9	0,44	6,45E-01	7,26E-01	NS
DGKZ	0,38	6,82E-01	7,63E-01	NS
DPP10	0,37	6,92E-01	7,69E-01	NS
MMP7	0,36	6,97E-01	7,70E-01	NS
MCP-2	0,35	7,04E-01	7,73E-01	NS
PADI2	0,33	7,20E-01	7,86E-01	NS
KLRD1	0,32	7,24E-01	7,86E-01	NS
CXADR	0,23	7,96E-01	8,58E-01	NS
IL5	0,22	8,00E-01	8,58E-01	NS
CXCL10	0,22	8,06E-01	8,59E-01	NS
NTF4	0,20	8,18E-01	8,67E-01	NS
CD40	0,19	8,23E-01	8,67E-01	NS
PTH1R	0,16	8,52E-01	8,92E-01	NS
CD28	0,13	8,81E-01	9,10E-01	NS
CRTAM	0,12	8,87E-01	9,10E-01	NS
ITGA11	0,12	8,87E-01	9,10E-01	NS
TRAIL	0,12	8,89E-01	9,10E-01	NS
CD28	0,07	9,29E-01	9,44E-01	NS
CXCL9	0,05	9,49E-01	9,60E-01	NS
IL5	0,04	9,61E-01	9,66E-01	NS
MCP-3	0,03	9,69E-01	9,69E-01	NS