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Differences in Biomarkers of Inflammation Between Novel Subgroups of Recent-Onset Diabetes

Short running title: Inflammation and novel diabetes subgroups

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Abstract

A novel clustering approach identified five subgroups of diabetes with distinct progression trajectories of complications. We hypothesized that these subgroups differ in multiple biomarkers of inflammation. Serum levels of 74 biomarkers of inflammation were measured in 414 individuals with recent adult-onset diabetes from the German Diabetes Study (GDS) allocated to five subgroups based on data-driven analysis. Pairwise differences between subgroups for biomarkers were assessed with generalized linear mixed models before (model 1) and after adjustment (model 2) for the clustering variables. Participants were assigned to five subgroups: severe autoimmune diabetes (SAID, 21%), severe insulin-deficient diabetes (SIDD, 3%), severe insulin-resistant diabetes (SIRD, 9%), mild obesity-related diabetes (MOD, 32%) and mild age-related diabetes (MARD, 35%). In model 1, 23 biomarkers showed ≥1 pairwise difference between subgroups (Bonferroni-corrected $p<0.0007$). Biomarker levels were generally highest in SIRD and lowest in SIDD. All 23 biomarkers correlated with ≥1 of the clustering variables. In model 2, three biomarkers (CASP-8, EN-RAGE, IL-6) showed at least one pairwise difference between subgroups (e.g. lower CASP8, EN-RAGE and IL-6 in SIDD vs. all other subgroups, all $p<0.0007$). Thus, novel diabetes subgroups show multiple differences in biomarkers of inflammation, underlining a prominent role of inflammatory pathways in particular in SIRD.

Diabetes mellitus is a multifactorial disease characterized by a complex combination of different but only partly understood etiologies (1). This heterogeneity is not adequately reflected by the current classification into the main forms of type 1 diabetes, type 2 diabetes and gestational diabetes. In particular, the diagnosis of type 2 diabetes usually comprises forms of diabetes that cannot be assigned to any other specific diabetes types. This classic classification fails to consider possible differences in disease mechanisms, does not allow identification of people with different risk of developing complications and precludes stratification of care and treatment regimens.

Recently, a data-driven cluster analysis of Scandinavian cohorts identified five diabetes subgroups (clusters) based on six variables: age at diagnosis, body mass index (BMI), HbA1c, homoeostasis model assessment-2 estimates of beta-cell function and insulin resistance (HOMA2-B and HOMA2-IR), and glutamate decarboxylase antibodies (GADA) (2). The five subgroups were validated in the German Diabetes Study (GDS) (3) and other cohort studies (3, 4) and multinational trial populations (5). These studies demonstrated that the five diabetes subgroups have distinct progression trajectories of diabetes-related complications (2, 3, 5), which have been related to differences in clinical, metabolic and genetic characteristics (3).

Given the established role of inflammatory processes in the development of diabetes-related complications (6-10), potential differences in biomarkers of inflammation between diabetes subgroups might also contribute to differences in outcomes. However, biomarkers of inflammation investigated in this context have been limited to high-sensitivity C-reactive protein (hsCRP) (3) so that there is an obvious need to investigate in further detail whether differences in other biomarkers of inflammation exist between these subgroups. Therefore, this study aimed (i) to comprehensively characterize differences in biomarkers of inflammation between the diabetes subgroups as described by Ahlqvist et al. and Zaharia et al. using a multimarker panel of serum protein biomarkers (6) and (ii) to investigate if these differences are fully or partly independent of the aforementioned six clustering variables.

RESEARCH DESIGN AND METHODS

Study Population

This study is based on data from the GDS cohort (11), an ongoing prospective observational cohort study investigating the natural course of metabolic alterations and the development of chronic diabetic complications (ClinicalTrial.gov, number NCT01055093). The GDS was approved by the ethics committee of Heinrich Heine University, Düsseldorf, Germany (ref. 4508). The study is performed in accordance with the Declaration of Helsinki. All participants provided written informed consent.

The GDS enrols individuals with recent-onset diabetes (known diabetes duration ≤1 year) aged 18-69 years and glucose-tolerant individuals serving as controls. Diabetes was diagnosed in accordance with the guidelines of the American Diabetes Association (12). Study design and cohort profile of the GDS were described in detail before (11). Exclusion criteria are any secondary forms of diabetes, poor glycaemic control (HbA1c >9.0%/75 mmol/mol), current pregnancy, acute or severe chronic cardiac, hepatic, renal or psychiatric diseases, active cancer, anemia; and acute infections, leukocytosis, immunosuppressive therapy, autoimmune diseases and infection with human immunodeficiency virus (11).

This cross-sectional analysis was based on the consecutive sampling of 504 participants with diabetes who entered the GDS cohort between September 2005 and December 2011, of whom 414 had available data for both biomarkers of inflammation and cluster assignment to one of the five to diabetes subgroups as described (2, 3). As shown in **Figure S1**, the sample represents a subsample of the GDS previously used in our analysis of diabetes subgroups and complications over a 5-year follow-up period, which additionally comprised study participants with baseline examinations until September 2018 (3).

Phenotyping and Laboratory Measurements

The study design included a structured interview, anthropometry, blood sampling and measurement of metabolic variables and autoantibodies (GADA, islet-cell autoantibodies [ICA]) as reported before (2, 11). HOMA2-B and HOMA2-IR were calculated using the HOMA2 calculator from the University of Oxford based on fasting C-peptide and fasting glucose concentrations (13). Anti-inflammatory medication was paused a minimum of seven days prior to blood sampling.

Biomarkers of inflammation were measured in serum of fasting participants at baseline using the Inflammation Panel from OLINK Proteomics (Uppsala, Sweden) as described (14). This assay is based on proximity extension assay (PEA) technology and allows the simultaneous measurement of 92 protein biomarkers covering pro- and anti-inflammatory cytokines, chemokines, growth factors and factors involved in acute inflammatory and immune responses, angiogenesis, fibrosis and endothelial activation (6). Therefore, "biomarkers of inflammation" in this manuscript refers to the biomarkers from this panel although some of them may also be considered metabolic biomarkers or biomarkers also reflecting other pathways.

The assay provides a relative quantification of protein concentrations that are given as normalized protein expression (NPX) values. These biomarker levels are comparable in their distribution to log2-transformed protein concentrations. The normalization procedure is required to convert cycle threshold values from the quantitative polymerase chain reaction assay to relative protein concentrations.

All biomarkers are listed in **Table S1** together with UniProt numbers, gene names, intra-assay coefficients of variation (CV) and inter-assay CVs. As described (14), the calculation of intra- and inter-assay CVs was based on three control sera measured in duplicates on each plate (n=7). Due to technical issues with the assay for brain-derived neurotrophic factor (BDNF), data for this biomarker were not reported. Further 17 biomarkers were excluded because they had more than 25% of values below the limit of detection. We had a priori defined a threshold level of

20% for intra- and inter-assay CVs as a criterion for exclusion of biomarkers but as reported before (14) intra- and inter-assay CVs ranged between 0.4-12.5% and 0.9-11.6%, respectively, so that no biomarkers needed to be excluded and 74 biomarkers remained for analysis. Two of these 74 biomarkers (IL-6, IL-18) had been measured before using ELISAs (15, 16). Log_2-transformed absolute protein concentrations (ELISA) and NPX (PEA) were highly correlated for both IL-6 (r=0.89, P<0.0001) and IL-18 (r=0.74, P<0.0001) (**Figure S2**),

Statistical Analysis

The allocation to previously defined diabetes subgroups (clusters) was performed based on age at diagnosis, BMI, HbA1c, HOMA2-B, HOMA2-IR and GADA as described before (2, 3). The cluster assignment was performed in the previously described sample from the GDS cohort (3) according to the sex-specific classification rules from Ahlqvist et al. (2) using the nearest centroid approach so that every individual was assigned to one out of five predefined clusters, i.e. severe autoimmune diabetes (SAID), severe insulin-deficient diabetes (SIDD), severe insulin-resistant diabetes (SIRD), mild obesity-related diabetes (MOD) and mild age-related diabetes (MARD). All individuals tested positive for GADA were allocated to the SAID cluster. We did not develop novel or updated classification rules in the GDS as our previous study primarily aimed at evaluation of specific features of the originally introduced subgroups (3) and because of the possible selection bias in recruitment, whereas the initial clustering algorithm was developed using a population-based sample (2). Differences in subgroup proportions between this sample and excluded individuals from the previously described sample from the GDS cohort (3) were analyzed using the χ^2 test.

Data are presented as median (25^{th} percentile; 75^{th} percentile) or percentages (%). Differences in the clinical characteristics of the study population according to diabetes subgroups were tested with Wilcoxon-Mann-Whitney, χ^2 test and Fisher's exact test. P values <0.05 were considered to indicate statistically significant differences.

Overall differences in biomarkers of inflammation between diabetes subgroups were analyzed using the Kruskal-Wallis test. Bonferroni-corrected *P* values <0.0007 (0.05/74) indicated significant differences.

Pairwise differences between means of biomarkers of inflammation across diabetes subgroups were estimated using generalized linear mixed models. The GLIMMIX procedure fits statistical models to unbalanced data and provides the capability to account for unequal residual variances between diabetes subgroups. Data were analyzed without (model 1) and with adjustment for age at diagnosis, BMI, HbA1c, HOMA2-B, HOMA2-IR and GADA (model 2; all covariables entered the model as continuous variables). *P* values of pairwise mean differences were adjusted for pairwise multiple comparisons using the Tukey-Kramer procedure (based on 10 different combinations of subgroups) and additionally for the total number of biomarkers using the Bonferroni procedure. A Bonferroni-corrected Tukey-Kramer *P* value <0.0007 was considered statistically significant (α=0.05/74=0.0007).

Correlations between inflammation-related biomarkers showing differences between subgroups in model 1 and the six aforementioned clustering variables were estimated using Spearman's rank correlation coefficients and corresponding *P* values.

The aforementioned statistical analyses were conducted using SAS version 9.4 (SAS Institute, Cary, NC, USA).

Exploratory principal component analysis (PCA) was performed in RStudio (Version 4.0.2) using the factoextra package to compare the separation of the diabetes subgroups (i) based on clustering variables and (ii) based on biomarkers of inflammation. As GADA is the sole criterion for the allocation to SAID, we focused on the remaining clustering variables to visualize clustering of the SIDD, SIRD, MOD and MARD subgroups.

In a causal inference analysis, we applied the two-sample Mendelian randomization (MR) method to evaluate the bi-directional causal relationship between biomarkers, which showed significant differences between diabetes subgroups, and type 1 diabetes, type 2 diabetes and

their related complications. This analysis was performed using the MR-Base web interface (17). Instrumental variables (IVs) were extracted from a published genome-wide association study (GWAS) of SOMAscan-measured proteins (n=3301) (18). For diabetes and diabetes-related complications (angiopathy, retinopathy, neuropathy, renal complications), we used the latest GWAS for type 1 and type 2 diabetes of the MRC IEU OpenGWAS Project with 12,375 cases and 82,665 controls of the FinnGen Biobank (19). All datasets include observations in men and women of European ancestry. All IVs passed the threshold of $P<5x10e-8$. To select IVs, we first removed palindromic single nucleotide polymorphisms (SNPs) (defined as SNPs with A/T or G/C) alleles and with minor allele frequencies close to 0.5 as the effect allele will be ambiguous. Then, we replaced these SNPs with proxies with a minimum linkage disequilibrium R^2 value of 0.8 when available and allowed palindromic SNPs with a minor allele frequency threshold up to 0.3. Finally, to identify the independent signals among correlated SNPs, we clumped the SNPs by removing SNPs in linkage disequilibrium with the lead SNP using the R^2 cut-off 0.001. A Bonferroni-corrected *P* value <0.005 was considered statistically significant ($\alpha=0.05/10=0.005$).

Data and Resource Availability

The data are subject to national data protection laws. Therefore, data cannot be made freely available in a public repository. However, data can be requested through an individual project agreement with the Steering Committee of the GDS (speaker: Michael Roden, michael.roden@ddz.de).

RESULTS

Study Population

As shown in **Table 1**, each of the 414 study participants was assigned to one of the five subgroups based on the clustering approach described before (2, 3): SAID, 21% (n=87); SIDD, 3% (n=12); SIRD, 9% (n=39); MOD, 32% (n=133); and MARD, 35% (n=143). The proportions of subgroups in our sample did not differ from those in the previously described larger sample from the GDS cohort (SAID, 22%; SIDD, 3%; SIRD, 11%, MOD, 29%, MARD, 35%; *P*=0.377 for the difference between included and excluded individuals) (3). Participants in these subgroups differed not only in the clustering variables but also in all other variables tested except total cholesterol levels, known diabetes duration and prevalence of chronic kidney disease (**Table 1**).

Unadjusted Differences in Biomarkers of Inflammation Between Diabetes Subgroups

The overall comparison of biomarkers between diabetes subgroups showed differences in the serum levels of 27 out of 74 biomarkers of inflammation ($P<0.0007$ after accounting for multiple testing) (**Table S2**). Biomarker levels were generally highest in individuals from the SIRD subgroup, lowest in individuals from the SIDD subgroup and intermediate in the SAID, MOD and MARD subgroups (**Table S2**).

The pairwise comparison of biomarkers between diabetes subgroups without any adjustment for covariables (model 1) showed significant differences in 26 out of 74 biomarkers of inflammation after correction for pairwise multiple comparisons (based on 10 different combinations of subgroups) using the Tukey-Kramer test ($P<0.05$). Among these 26 biomarkers, 23 biomarkers showed at least one pairwise significant difference after additional Bonferroni correction ($P<0.0007$). **Figure 1A** graphically summarizes the effect estimates and corresponding *P* values of between-means pairwise comparisons of the 26 biomarkers while **Table S3** reports detailed results for all 74 biomarkers.

After Bonferroni correction, the largest number of pairwise differences between subgroups (at least 4) were observed for caspase-8 (CASP-8), CSF-1 (macrophage colony-stimulating factor

1), fibroblast growth factor-21 (FGF-21), HGF (hepatocyte growth factor) and interleukin-6 (IL-6). With respect to the number of biomarkers, the largest number of differences after Bonferroni correction were observed for the comparison of SIRD vs. MARD, SAID and SIDD with higher serum levels of 7, 8 and 9 biomarkers, respectively, in SIRD (all $P<0.0007$). MARD, MOD and SAID differed in 4-8 biomarker levels, but with different directions. Only one biomarker (CASP-8) was different in the comparisons of SIDD vs. SAID, MARD and MOD.

Differences in Biomarkers of Inflammation Between Diabetes Subgroups after Adjustment for Clustering Variables

The 26 biomarkers of inflammation with pairwise differences between diabetes subgroups were also correlated with at least one of the 6 clustering variables. **Figure 2** provides an overview of correlation coefficients and *P* values (see **Table S4** for detailed results). About two thirds of these biomarkers (between 17 and 19) correlated with age, BMI, HOMA2-B and HOMA2-IR with mostly positive correlation coefficients between 0.1 and 0.5. Correlations between biomarker levels and presence of GADA were positive for 4 and inverse for 10 biomarkers. Only 6 biomarkers correlated with HbA1c.

When we assessed differences in biomarker levels between subgroups with additional adjustment for these clustering variables (model 2), there were significant differences between subgroups for 13 biomarkers (Tukey-Kramer $P<0.05$). The largest number of pairwise differences between subgroups ($n=4$) were observed for caspase-8 (CASP-8), S100 calcium-binding protein A12 (EN-RAGE), interleukin-6 (IL-6) and tumor necrosis factor receptor superfamily member 9 (TNFRSF9/CD137). Regarding the number of biomarkers, most differences were found for the comparison of SIRD vs. SIDD with higher serum levels of 7 biomarkers in SIRD.

After additional adjustment for multiple testing, three biomarkers (CASP-8, EN-RAGE, IL-6) showed at least one pairwise difference between subgroups (**Figure 1B**, **Table S3**). **Figure 3** shows the unadjusted serum levels of these three biomarkers in each diabetes subgroup. CASP-8 levels were lower in SIDD than in SAID, SIRD, MARD and MOD; EN-RAGE levels were lower in SIDD than in MOD; and IL-6 levels were lower in SIDD than in MARD (all $P<0.0007$). None of the biomarkers differed between SAID, MOD and MARD after full adjustment.

Principal Component Analysis Using the Clustering Variables and Biomarkers of Inflammation

The first PCA using the clustering variables (except for GADA) indicated the largest difference between the SIRD and SIDD subgroups and an overlap between MOD and MARD (**Figure S3A**). The first two principal components (PC1, PC2) explained 64.7% of the variance. When using all 74 biomarkers of inflammation for the PCA, the separation was less pronounced, but again SIRD and SIDD were the subgroups with the best separation (**Figure S3B**). In the second analysis, PC1 and PC2 explained 29.1% of the variance (**Figure S4**).

Mendelian Randomization Analysis

We assessed the associations between genetically predicted levels of biomarkers which showed significant differences between diabetes subgroups (CASP-8, EN-RAGE, IL-6) and type 1 and type 2 diabetes and their related complications. No IVs were available for CASP-8 in the MR-Base platform. For EN-RAGE, a nominally significant association with renal complications in people with type 2 diabetes as observed (inverse-variance weighted $\beta=0.279$, $P=0.033$; **Table S5**), which did not remain significant after adjustment for multiple testing. No associations of genetically predicted EN-RAGE levels were detected with type 2 diabetes or the other tested complications in type 2 diabetes. There were also no associations of EN-RAGE with type 1

diabetes or any of the complications in people with type 1 diabetes (**Table S5**). For IL-6, there was no evidence for causal effects on any of the aforementioned outcomes (all $P \geq 0.087$; **Table S6**).

We also performed MR analyses to examine whether type 2 diabetes, type 1 diabetes or any of their complications had causal effects on EN-RAGE or IL-6 levels, but we found no evidence for this (all $P \geq 0.119$; **Table S7** and **Table S8**).

DISCUSSION

Diabetes subgroups identified using the clustering approach described by Ahlqvist et al. and validated by Zaharia et al. (2, 3, 20) showed multiple differences in biomarkers of inflammation. Biomarker levels were highest in the SIRD subgroup, which is characterized by pronounced insulin resistance, and lowest in the SIDD subgroup, which is characterized by severe insulin deficiency, and intermediate for the MOD, MARD and SAID subgroups. Although adjustment for clustering variables attenuated these differences, we were still able to identify three biomarkers (CASP-8, EN-RAGE, IL-6) that showed at least one pairwise difference between the subgroups in the adjusted model.

This study represents a novel comprehensive investigation of the relationship between biomarkers of inflammation and the five novel diabetes subgroups (clusters). Our previous analysis focused on metabolic features and comorbidities of these subgroups but already reported that hsCRP was higher in SIRD and MOD compared to the other subgroups (3). The identification of multiple differences in inflammation-related biomarkers validates the empirically derived classification based on age at diagnosis, BMI, metabolic parameters and autoimmunity. As all of these clustering variables correlate with subclinical inflammation, it is important to emphasize that the observed differences were at least partly independent of the

clustering variables. Of note, our study included only individuals with recent-onset diabetes which is more appropriate for drawing pathophysiological conclusions than investigating individuals with long-standing diabetes whose inflammatory profiles are substantially affected by diabetes-related complications and comorbidities.

Overall, the highest levels of biomarkers of inflammation were observed in SIRD, i.e. the diabetes subgroup characterized by the most pronounced insulin resistance. SIRD represents one of the smaller subgroups with 9% in our cohort and 10-17% in other European cohorts (2). However, this rather small subgroup is clinically important not only because of the high degree of insulin resistance but also because of higher prevalence and increased risk for diabetes-related comorbidities such as nephropathy (2, 3) or hepatic steatosis and early fibrosis (3). A close relationship between inflammation and insulin resistance has been previously established in multiple studies mostly in the context of obesity and dysfunctional adipose tissue (21). Inflammation-related processes in adipose tissue and spill-over of inflammatory biomarkers into the circulation have been recognized as important mechanisms possibly initiating abnormal hepatic glucose metabolism and type 2 diabetes (22). Of note, despite the similar degree of obesity, based on BMI, as MOD, SIRD may represent a state of dysfunctional adipose tissue, which is further supported by the higher prevalence of dyslipidemia. This along with hypertension would drive the onset of diabetes-related complications. After adjustment for clustering variables, SIRD had higher circulating levels of CASP-8 than SIDD. CASP-8 is a cytosolic cysteine protease that mediates programmed cell death. It is involved in beta-cell apoptosis in diabetes (23), but also in the activation of T, B, and natural killer cells and macrophage differentiation. It is not well understood which processes lead to the release of this protein into the circulation, but it is noteworthy that higher circulating CASP-8 levels were associated with a higher risk of type 2 diabetes and coronary events (24, 25).

SIDD represents the opposite extreme compared to SIRD with the most pronounced insulin deficiency and the lowest circulating levels of biomarkers of inflammation. Our exploratory PCA confirms the separation between SIRD and SIDD based on both the clustering variables and the biomarkers of inflammation. Three biomarkers were lower in SIDD than in all other subgroups after adjustment for clustering variables (CASP-8, IL-6, EN-RAGE). IL-6 is related to inflammation in adipose tissue. However, this cytokine has multiple sources and pleiotropic roles (26, 27). IL-6 is not only released by immune cells and adipocytes, but also by myocytes in response to exercise. In addition to proinflammatory properties, IL-6 mediates beneficial effects of exercise, stimulates insulin secretion through the release of glucagon-like peptide-1 (GLP-1) and may therefore represent an important cytokine that also counteracts metabolic stress and insulin resistance (28, 29). EN-RAGE signals through the receptor for advanced glycation endproducts and the Toll-like receptor, thereby triggering cytokine production, chemotaxis and increased oxidative stress (30). Higher circulating levels of EN-RAGE were associated with incident prediabetes and type 2 diabetes (31). Thus, it can be hypothesized that inflammation-related processes contribute to the development of diabetes more in the other subgroups than in SIDD, but this would require testing in a longitudinal study with blood samples also taken before the diagnosis of diabetes. It also appears that biomarkers of inflammation may not be relevant correlates of impaired beta-cell function although the role of inflammatory processes in beta-cell demise in individuals with diabetes is well established (32). Given that advanced age and most lifestyle-related and environmental risk factors of type 2 diabetes are triggers of subclinical inflammation (33), it is tempting to speculate that the aetiology of SIDD may have a stronger genetic component than SIRD, MARD and MOD. So far, there is only evidence for differential associations between the gene variant rs7903146 in the *TCF7L2* gene and the type 2 diabetes-related subgroups with the nominally highest effect size for SIDD (2).

Concerning the MARD, MOD and SAID subgroups, multiple differences in biomarkers of inflammation were seen in the unadjusted analysis, which, however, were abolished by adjustment for clustering variables. This observation suggests that these differences in inflammatory profiles are largely explained by age at diagnosis, anthropometry and metabolic variables. Of note, MARD and MOD represent subgroups of type 2 diabetes, whereas SAID mainly reflects type 1 diabetes. Previous studies that compared circulating levels of biomarkers of inflammation found higher levels for some cytokines and soluble adhesion molecules in patients with type 2 diabetes than in those with autoimmune diabetes but the distributions of biomarker concentrations overlapped widely (33, 34). In our study, levels of FGF-21, IL-6 and CDCP1 (CUB domain-containing protein 1) were lower in SAID compared to both MARD and MOD, but these differences were largely explained by adjustment for clustering variables. Therefore, biomarkers of inflammation do not appear to be better discriminators between type 1 diabetes and type 2 diabetes in the traditional classification system when age at diagnosis, anthropometry, metabolic variables and autoantibodies are taken into account. However, it needs to be acknowledged that the biomarker panel used in this study did not contain potentially important cytokines such as IL-1β, whereas biomarkers such as FGF-21 are more closely related to glucose and lipid metabolism than to inflammatory processes (35).

Future studies need to investigate to what extent differences in the profiles of inflammation-related biomarkers can explain the apparent differences between the diabetes subgroups regarding their risk to develop diabetes-related complications and comorbidities. Previous studies showed that SIRD was characterized by the highest risk of chronic kidney disease and different hepatic fat content and fibrosis (2, 3). A recent multimarker study found that mainly TNF receptor superfamily members showed robust associations with incident end-stage renal disease in people with diabetes (7), which is reflected by our finding that SIRD had the highest levels of TNFRSF9. In a recent report using the same biomarker panel investigated in the

current study, we showed multiple associations with eGFR in the GDS (14). Among the biomarkers associated with eGFR, 13 biomarkers showed differences between diabetes subgroups in the unadjusted model of the current study (**Figure 1A**) and six biomarkers (CD5 [T-cell surface glycoprotein CD5], CSF-1, CST5 [cystatin D], IL-12B [IL-12 subunit beta], TNFRSF9, uPA [urokinase-type plasminogen activator]) showed at least one between-subgroup difference after the adjustment for clustering variables (**Figure 1B**). The observations point towards a potential mediating role of these biomarkers in diabetes-related impairment of kidney function which, however, would have to be investigated using a prospective design in a larger study sample. SIRD also showed the highest levels of biomarkers such as IL-6, IL-17C, CCL20 (C-C motif chemokine 20), CASP-8 and CD5, which have been implicated in different stages of non-alcoholic fatty liver disease (NAFLD) (36-39) and merit further studies in this context.

In contrast, SIDD showed the strongest associations with retinopathy and diabetic sensorimotor polyneuropathy (DSPN) (2, 3). Inflammatory mechanisms and multiple biomarkers of inflammation are implicated in the development of DSPN (40, 41) so that an increased risk of DSPN in SIDD appears counterintuitive and needs to be replicated in additional cohorts before firm conclusions are possible. Finally, our data do not argue for biomarkers of inflammation as independent mediators of a differential risk of diabetes-complications between MOD, MARD and SAID beyond the age at diagnosis and metabolic variables.

To address the issue of causality in this context, we attempted a causal inference analysis using two-sample MR for CASP-8, EN-RAGE and IL-6. We found suggestive evidence for a causal effect of EN-RAGE on renal complications in people with type 2 diabetes but this finding was not significant after adjustment for multiple testing. Unfortunately, we were not able to check for horizontal pleiotropy as we only had two IVs. Moreover, the causal effect was mainly driven

by the SNP rs62143206 which is in trans with the protein-encoding gene. Overall, our analyses were limited by the availability of suitable IVs for estimating causal effects of these protein biomarkers on diabetes without and with complications (no SNP for CASP-8, only two SNPs each for EN-RAGE and IL-6). In the absence of larger GWAS for these protein biomarkers, our null findings need to be interpreted with caution. Additional IVs that could explain a larger proportion of variance in biomarker levels would increase the statistical power of such MR analyses and help to reveal potential causal effects.

It is important to note that our cross-sectional study focused on differences in biomarkers of inflammation and diabetes subgroups at only one time-point during the first year after the diagnosis of diabetes. We have previously shown in the GDS that the cluster allocation can change during the first five years of follow-up and that these changes were mainly related to changes in glycaemia and lipid levels (3). Studies comprising multiple measurements of clinical variables and biomarkers are needed to model trajectories of clustering variables and biomarkers of inflammation (see [42] and [43] for examples) ideally before and after the diagnosis of diabetes (44). These data would allow an analysis of the temporal relationships between changes in clustering variables and changes in biomarker levels allowing further insights into aetiological pathways which might differ between the diabetes subgroups.

Our study has several strengths and limitations. A major strength is the unique cohort of people with recent-onset diabetes so that differences in biomarkers of inflammation between diabetes subgroups can be assessed before potential confounding by long-standing hyperglycemia and high prevalence of complications. Moreover, the use of a multi-marker approach implicated comprehensive phenotyping of inflammation-related biomarkers which is important because multiple parallel pathomechanisms can be expected to contribute to the pathophysiology of diabetes and its complications. Limitations of our study include the cross-sectional design and

the low absolute number of individuals with SIDD and SIRD. Differences in the proportions of individuals in each subgroup compared to other European cohorts may partly be explained by the specific inclusion and exclusion criteria (e.g. limited age range, upper HbA1c level, overrepresentation of individuals with type 1 diabetes) of the GDS. The exclusion of individuals with poor glycemic control might have led to the exclusion of the most extreme SIDD cases and thus to an underestimation of the differences in biomarkers between the SIDD and other subgroups. The oversampling of individuals with type 1 diabetes may also have resulted in a larger proportion of ICA-positive individuals among the GADA-negative subgroups than in other cohorts (45, 46). The multimarker panel contained a range of biomarkers of inflammation but interesting proteins such as further members of the IL-1 family were not included. The procedure to correct for multiple testing accounting for both the number of between-subgroup comparisons and the number of biomarkers may be too conservative given the correlation structure between the biomarkers. Therefore, the number of biomarkers that differ between diabetes subgroups may be underestimated. However, such a cautious approach appears preferable in the absence of external validation of our results. Not only should our results be replicated in external cohorts with participants with recent-onset diabetes, but future studies should also assess the predictive value of biomarkers of inflammation for the risk of complications of diabetes in prospective analyses and test to what extent these biomarkers may contribute to differences in diabetes-related complications among novel diabetes subgroups (e.g. using mediation analysis). Causal inference analyses such as Mendelian randomization studies can help to assess the role of predictive biomarkers for disease etiology, but these analyses were limited by the scarcity of suitable IVs and thus low statistical power. Future studies might also revisit the definition of the SAID subgroup which is currently based on GADA positivity only. The presence of ICA in some individuals from the other four subgroups suggests that the measurement of multiple autoantibodies may be better suited to assess

autoimmunity as a clustering criterion. Finally, our study sample consisted mainly of people of European descent and consequently, results are not generalizable to other ethnicities.

In conclusion, our study identified multiple differences in biomarkers of inflammation between novel subgroups of diabetes. Circulating levels of biomarkers of inflammation were highest in SIRD and lowest in SIDD. Differences between subgroups remained significant for three biomarkers even after adjustment for clustering variables. Of note, there was no clear separation of SAID, reflecting type 1 diabetes, from MARD and MOD currently classified as type 2 diabetes with regard to biomarkers of inflammation. The link between high levels of inflammation-related biomarkers and pronounced insulin resistance points to a particular contribution of inflammatory processes to the SIRD subgroup. Future studies are warranted to investigate which of the biomarkers identified in this study may explain differences in the risk of complications between the subgroups.

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Conflict of Interest

The authors declare that there is no duality of interest associated with this manuscript.

Author Contributions

C.H. and H.M. designed the study. C.H., O.-P.Z., Y.K., K.B., S.T., V.B., J.S. and M.R. contributed data. C.H. and H.M. drafted the analysis plan. H.M. performed the statistical analysis. C.H., K.S., J.M.R., M.A.E., B.W.C.B. and M.W. contributed to the statistical analysis. C.H., H.M. and J.M.R. interpreted data. M.A.E., W.R., M.W. and M.R. contributed to data interpretation. C.H. wrote the manuscript. H.M., J.M.R. and M.R. contributed to the draft of the manuscript. All authors reviewed and edited the manuscript and approved of its submission.

Guarantor Statment

C.H. and H.M. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Table 1 - Clinical characteristics of the study population according to diabetes subgroup allocation

Characteristic	SAID	SIDD	SIRD	MOD	MARD	*P*
N (% of study sample)	87 (21%)	12 (3%)	39 (9%)	133 (32%)	143 (35%)	
Age, years	34.5 (25.9; 46.2)	46.3 (33.4; 52.9)	56.4 (50.7; 64.8)	43.9 (35.3; 53.1)	59.8 (53.8; 64.5)	**<0.0001**
Sex (men/women), %	59.7/40.3	91.7/8.3	64.1/35.9	57.1/42.9	71.3/28.7	**0.02**
BMI, kg/m^2	24.0 (21.9; 26.5)	27.1 (24.5; 29.0)	34.9 (31.1; 37.4)	33.7 (30.0; 38.5)	27.3 (25.4; 29.9)	**<0.0001**
Waist circumference, cm	86.0 (76.5; 96.0)	94.0 (82.2; 106.0)	114.0 (106.0; 122.0)	109.5 (98.2; 119.0)	96.1 (90.0; 104.0)	**<0.0001**
HOMA2-B	47.8 (34.9; 69.3)	35.8 (24.1; 45.2)	165.9 (136.5; 187.6)	91.2 (70.4; 119.9)	81.6 (63.3; 105.9)	**<0.0001**
HOMA2-IR	1.0 (0.7; 1.3)	1.4 (1.0; 2.7)	4.4 (3.8; 5.5)	2.7 (1.9; 3.4)	1.8 (1.4; 2.3)	**<0.0001**
Total cholesterol, mg/dl	185.0 (163.5; 213.5)	196.5 (190.5; 200.0)	203.5 (173.5; 228.5)	198.5 (167.5; 231.5)	197.5 (173.5; 226.5)	0.13
Triglycerides, mg/dl	74.3 (53.4; 109.0)	100.1 (66.1; 191.6)	176.6 (110.5; 243.6)	141.3 (91.5; 206.0)	109.6 (83.0; 152.2)	**<0.0001**
HbA1c, %	6.4 (6.0; 7.1)	8.8 (8.0; 10.2)	6.0 (5.8; 6.3)	6.4 (5.8; 7.0)	6.1 (5.8; 6.5)	**<0.0001**
HbA1c, mmol/mol	46.4 (42.1; 54.1)	72.7 (63.4; 87.4)	42.1 (39.9; 45.3)	46.4 (39.9; 53.0)	43.1 (39.9; 47.5)	**<0.0001**
eGFR (ml/min per 1.73m^2)	101.0 (86.7; 110.3)	102.6 (92.1; 114.8)	78.6 (68.7; 88.8)	95.4 (85.3; 108.3)	87.6 (75.9; 97.7)	**<0.0001**
CKD, %	0	0	7.9	3.0	6.4	0.05
Known diabetes duration, days	162 (123; 255)	113.0 (49.5; 192.0)	197 (124; 279)	179 (122; 176)	194 (132; 266)	0.52
Hypertension, %	25.6	41.7	78.9	61.0	75.7	**<0.0001**
Antihypertensive medication, %	10.3	16.7	64.1	45.1	51.0	**<0.0001**
Lipid-lowering medication, %	2.3	8.3	30.7	9.8	25.2	**<0.0001**
Glucose-lowering medication (insulin/metformin/none/other), %	77/7/11/5	50/25/8/17	0/67/25/8	19/33/38/10	10/35/49/6	**<0.0001**
GADA >0.9 U/mL, %	100	0	0	0	0	**<0.0001**
ICA >20 JDF units, %	83.9	8.3	2.6	6.0	2.8	**<0.00001**

Data are presented as median (25th percentile; 75th percentile) or percentages (%). The frequency of missing data is <2% for each variable.

CKD, chronic kidney disease; GADA, glutamic acid decarboxylase autoantibodies; HOMA2-B, homoeostatic assessment model for β-cell function; HOMA2-IR, homoeostatic assessment model for insulin resistance; ICA, islet-cell autoantibodies; JDF, Juvenile Diabetes Foundation; MARD,

moderate age-related diabetes; MOD, moderate obesity-related diabetes; SAID, severe autoimmune diabetes; SIDD, severe insulin-deficient diabetes; SIRD, severe insulin-resistant diabetes.

CKD was defined by eGFR <60 mL/min/1.73 m^2 (calculated using the CKD-EPI equation based on creatinine and cystatin C).

Figure 1A – Unadjusted pairwise differences between diabetes subgroups (model 1)

The heatmap shows effect estimates and corresponding *P* values of pairwise comparisons of the 26 biomarkers with at least one significant pairwise difference after correction for pairwise and/or multiple comparisons (model 1). Extended names of biomarkers are given in Table S1. Full results are given in Table S3.
Positive/negative effect estimates result from higher/lower biomarker levels in the diabetes subgroup named first in the respective comparison.
*Tukey-Kramer corrected $P<0.05$; **Bonferroni-corrected Tukey-Kramer $P<0.0007$.
Biomarkers are ordered alphabetically from top to bottom.

Figure 1B – Pairwise differences between diabetes subgroups adjusted for clustering variables (model 2)

The heatmap shows effect estimates and corresponding *P* values of pairwise comparisons of the 26 biomarkers with at least one significant pairwise difference after correction for pairwise and/or multiple comparisons (model 2). Extended names of biomarkers are given in Table S1. Full results are given in Table S3.
Positive/negative effect estimates result from higher/lower biomarker levels in the diabetes subgroup named first in the respective comparison.
*Tukey-Kramer corrected $P<0.05$; **Bonferroni-corrected Tukey-Kramer $P<0.0007$.
Biomarkers are ordered alphabetically from top to bottom.

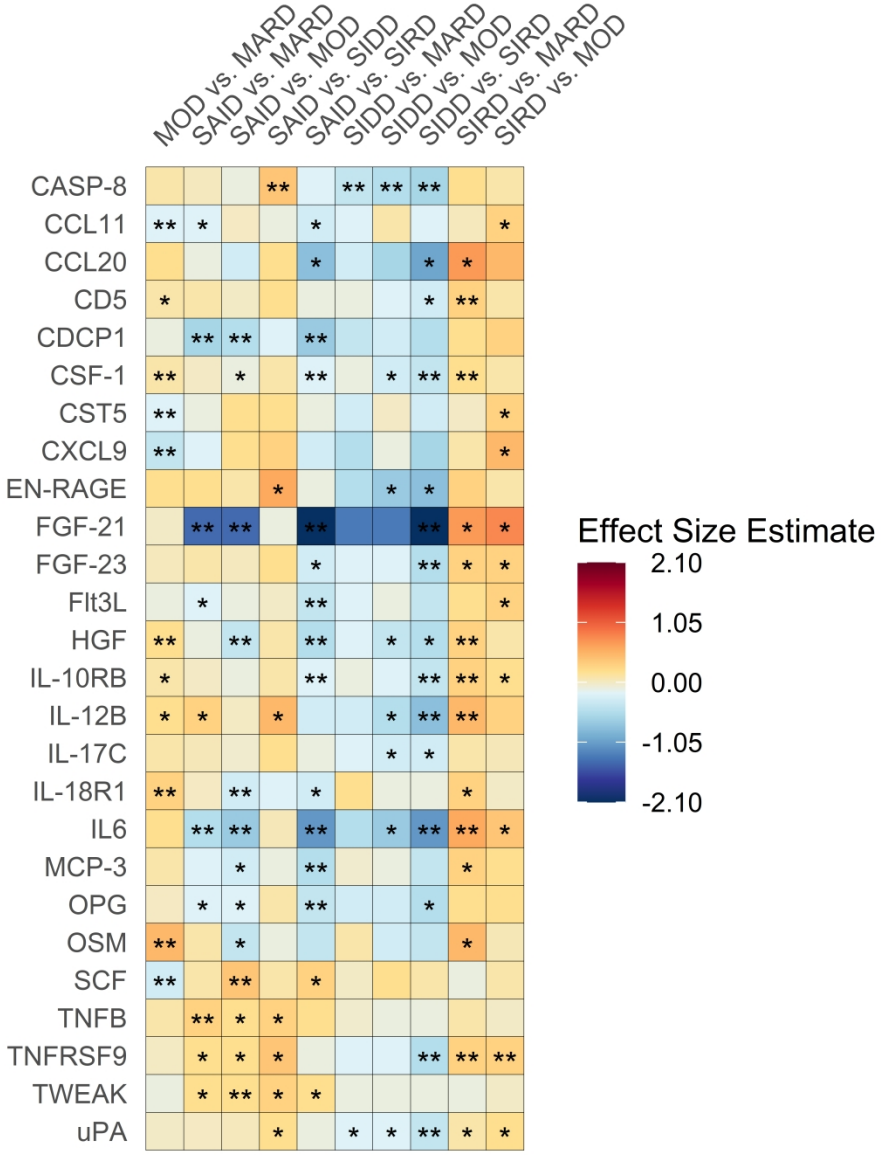
Figure 2 – Correlations between biomarkers of inflammation and clustering variables in the total study sample

Correlations with the clustering variables were assessed for the 26 biomarkers of inflammation with pairwise differences between diabetes subgroups.
Age, age at diagnosis; GADA, glutamic acid decarboxylase autoantibodies; HOMA2-B, homoeostatic assessment model for β-cell function; HOMA2-IR, homoeostatic assessment model for insulin resistance.
Extended names of biomarkers are given in Table S1.

Figure 3 – Serum concentrations (in normalized protein expression units [NPX]) of the three biomarkers of inflammation in the diabetes subgroups showing at least one pairwise difference between subgroups after adjustment for clustering variables

Data are shown as median ± 25^{th}/75^{th} percentiles.

CASP8, caspase-8; EN-RAGE, S100 calcium-binding protein A12; IL-6, interleukin-6.



Unadjusted pairwise differences between diabetes subgroups (model 1)

The heatmap shows effect estimates and corresponding P values of pairwise comparisons of the 26 biomarkers with at least one significant pairwise difference after correction for pairwise and/or multiple comparisons (model 1). Extended names of biomarkers are given in Table S1. Full results are given in Table S3.
Positive/negative effect estimates result from higher/lower biomarker levels in the diabetes subgroup named first in the respective comparison.
*Tukey-Kramer corrected P<0.05; **Bonferroni-corrected Tukey-Kramer P<0.0007.
Biomarkers are ordered alphabetically from top to bottom.

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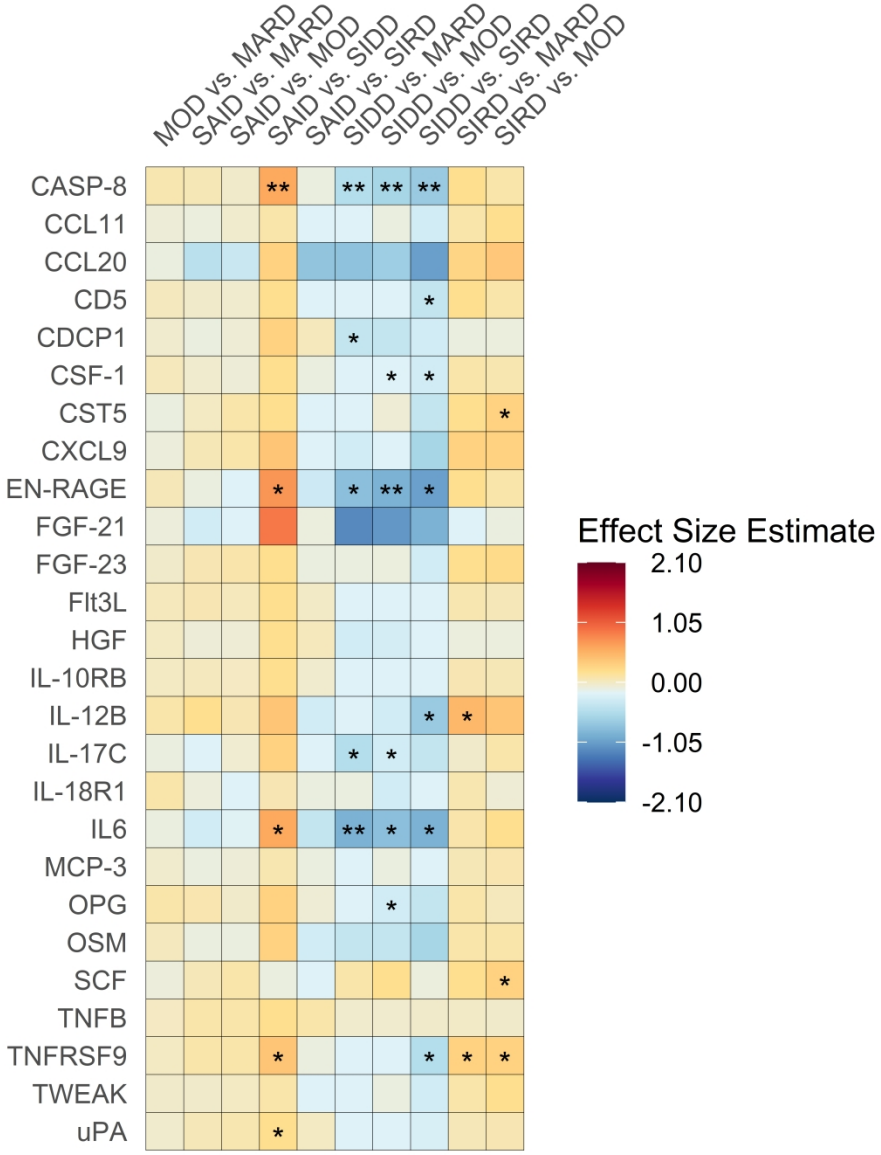

Pairwise differences between diabetes subgroups adjusted for clustering variables (model 2)

The heatmap shows effect estimates and corresponding P values of pairwise comparisons of the 26 biomarkers with at least one significant pairwise difference after correction for pairwise and/or multiple comparisons (model 2). Extended names of biomarkers are given in Table S1. Full results are given in Table S3.
Positive/negative effect estimates result from higher/lower biomarker levels in the diabetes subgroup named first in the respective comparison.
*Tukey-Kramer corrected P<0.05; **Bonferroni-corrected Tukey-Kramer P<0.0007.
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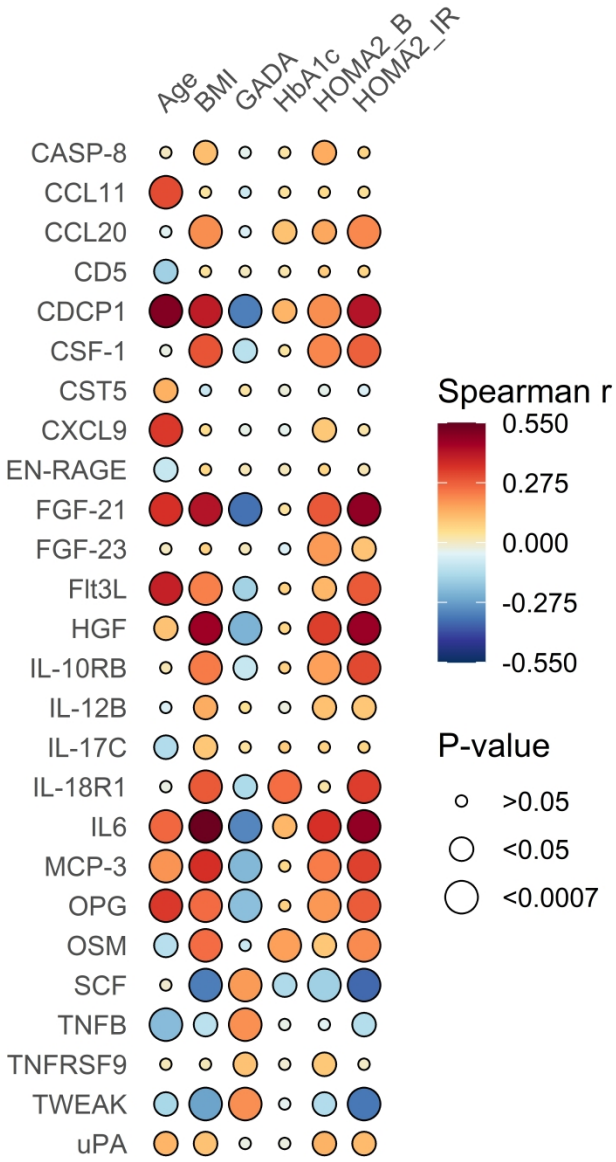

Correlations between biomarkers of inflammation and clustering variables in the total study sample

Correlations with the clustering variables were assessed for the 26 biomarkers of inflammation with pairwise differences between diabetes subgroups.
Age, age at diagnosis; GADA, glutamic acid decarboxylase autoantibodies; HOMA2-B, homoeostatic assessment model for β-cell function; HOMA2-IR, homoeostatic assessment model for insulin resistance.
Extended names of biomarkers are given in Table S1.

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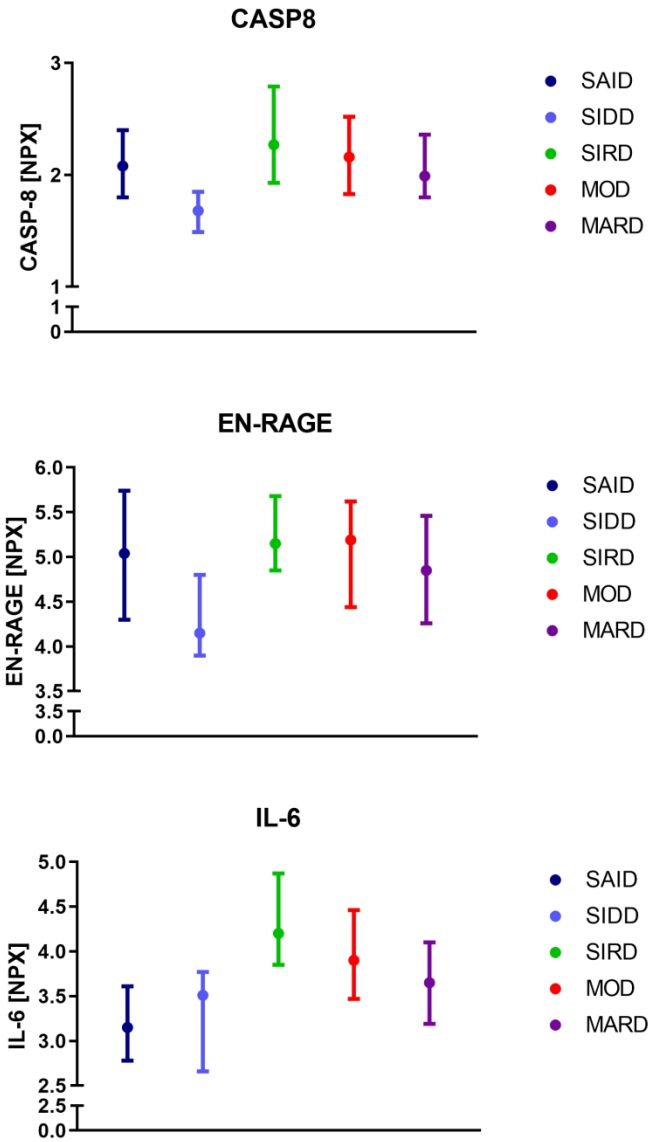


Serum concentrations (in normalized protein expression units [NPX]) of the three biomarkers of inflammation in the diabetes subgroups showing at least one pairwise difference between subgroups after adjustment for clustering variables

Data are shown as median ± 25th/75th percentiles.
CASP8, caspase-8; EN-RAGE, S100 calcium-binding protein A12; IL-6, interleukin-6.

34x56mm (1200 x 1200 DPI)

Table S1 - Biomarkers in the OLINK Inflammation panel and assay characteristics in the German Diabetes Study

Assay ID	Biomarker	Full name	UniProt No	Gene symbol	Intra-assay CV (%)	Inter-assay CV (%)
101_IL-8	IL-8	Interleukin-8	P10145	*CXCL8*	1.1	1.6
102_VEGF-A	VEGF-A	Vascular endothelial growth factor A	P15692	*VEGFA*	0.8	1.1
103_BDNF*	BDNF	Brain-derived neurotrophic factor	P23560	*BDNF*	n/a	n/a
105_MCP-3	MCP-3	Monocyte chemotactic protein 3 (CCL7)	P80098	*CCL7*	4.7	8.2
106_GDNF	GDNF	Glial cell line-derived neurotrophic factor	P39905	*GDNF*	9.6	8.3
107_CDCP1	CDCP1	CUB domain-containing protein 1	Q9H5V8	*CDCP1*	3.3	5.1
108_CD244	CD244	Natural killer cell receptor 2B4	Q9BZW8	*CD244*	1.2	2.2
109_IL-7	IL-7	Interleukin-7	P13232	*IL7*	1.2	2.5
110_OPG	OPG	Osteoprotegerin	O00300	*TNFRSF11B*	0.5	1.0
111_LAP TGF-beta-1	LAP TGFβ1	Latency-associated peptide transforming growth factor beta-1	P01137	*TGFB1*	0.8	1.6
112_uPA	uPA	Urokinase-type plasminogen activator	P00749	*PLAU*	0.6	1.4
113_IL-6	IL-6	Interleukin-6	P05231	*IL6*	1.9	4.0

114_IL-17C	IL-17C	Interleukin-17C	Q9P0M4	*IL17C*	7.0	6.0
115_MCP-1	MCP-1	Monocyte chemotactic protein 1 (CCL2)	P13500	*CCL2*	0.8	1.9
116_IL-17A	IL-17A	Interleukin-17A	Q16552	*IL17A*	12.5	11.5
117_CXCL11	CXCL11	C-X-C motif chemokine 11	O14625	*CXCL11*	0.8	2.5
118_AXIN1	Axin-1	Axin-1	O15169	*AXIN1*	5.1	10.4
120_TRAIL	TRAIL	TNF-related apoptosis-inducing ligand (TNFSF10)	P50591	*TNFSF10*	0.7	1.6
121_IL-20RA**	IL-20RA	Interleukin-20 receptor subunit alpha	Q9UHF4	*IL20RA*	n/a	n/a
122_CXCL9	CXCL9	C-X-C motif chemokine 9	Q07325	*CXCL9*	0.9	2.6
123_CST5	CST5	Cystatin D	P28325	*CST5*	0.9	2.1
124_IL-2RB**	IL-2RB	Interleukin-2 receptor subunit beta	P14784	*IL2RB*	n/a	n/a
125_IL-1 alpha**	IL-1α	Interleukin-1 alpha	P01583	*IL1A*	n/a	n/a
126_OSM	OSM	Oncostatin-M	P13725	*OSM*	1.4	3.7
127_IL-2**	IL-2	Interleukin-2	P60568	*IL2*	n/a	n/a
128_CXCL1	CXCL1	C-X-C motif chemokine 1	P09341	*CXCL1*	0.7	1.4
129_TSLP**	TSLP	Thymic stromal lymphopoietin	Q969D9	*TSLP*	n/a	n/a

130_CCL4	CCL4	C-C motif chemokine 4	P13236	*CCL4*	0.9	1.6
131_CD6	CD6	T cell surface glycoprotein CD6 isoform	Q8WWJ7	*CD6*	1.5	3.6
132_SCF	SCF	Stem cell factor (c-Kit-ligand)	P21583	*KITLG*	0.4	1.0
133_IL-18	IL-18	Interleukin-18	Q14116	*IL18*	0.9	2.9
134_SLAMF1	SLAMF1	Signaling lymphocytic activation molecule (SLAM)	Q13291	*SLAMF1*	11.6	11.5
135_TGF-alpha	TGFα	Transforming growth factor alpha	P01135	*TGFA*	2.2	3.6
136_MCP-4	MCP-4	Monocyte chemotactic protein 4 (CCL13)	Q99616	*CCL13*	1.9	3.9
137_CCL11	Eotaxin	Eotaxin (CCL11)	P51671	*CCL11*	0.8	1.8
138_TNFSF14	TNFSF14	Tumor necrosis factor ligand superfamily member 14 (LIGHT)	O43557	*TNFSF14*	1.0	2.7
139_FGF-23	FGF-23	Fibroblast growth factor 23	Q9GZV9	*FGF23*	8.7	11.4
140_IL-10RA	IL-10RA	Interleukin-10 receptor subunit alpha	Q13651	*IL10RA*	7.5	9.8
141_FGF-5	FGF-5	Fibroblast growth factor 5	Q8NF90	*FGF5*	11.7	9.3
142_MMP-1	MMP-1	Matrix metalloproteinase-1	P03956	*MMP1*	0.4	0.9
143_LIF-R	LIF-R	Leukemia inhibitory factor receptor	P42702	*LIFR*	1.4	4.6
144_FGF-21	FGF-21	Fibroblast growth factor 21	Q9NSA1	*FGF21*	1.2	7.8

145_CCL19	CCL19	C-C motif chemokine 19	Q99731	*CCL19*	0.8	2.5
148_IL-15RA	IL-15RA	Interleukin-15 receptor subunit alpha	Q13261	*IL15RA*	11.7	9.6
149_IL-10RB	IL-10RB	Interleukin-10 receptor subunit beta	Q08334	*IL10RB*	1.1	1.2
150_IL-22 RA1**	IL-22RA1	Interleukin-22 receptor subunit alpha-1	Q8N6P7	*IL22RA1*	n/a	n/a
151_IL-18R1	IL-18R1	Interleukin-18 receptor 1	Q13478	*IL18R1*	1.0	2.1
152_PD-L1	PD-L1	Programmed cell death 1 ligand 1	Q9NZQ7	*CD274*	1.7	2.7
153_Beta-NGF	Beta-NGF	Beta-nerve growth factor	P01138	*NGF*	3.2	5.6
154_CXCL5	CXCL5	C-X-C motif chemokine 5	P42830	*CXCL5*	0.6	1.4
155_TRANCE	TRANCE	TNF-related activation-induced cytokine (TRANCE, TNFSF11, RANKL, OPGL)	O14788	*TNFSF11*	2.0	5.4
156_HGF	HGF	Hepatocyte growth factor	P14210	*HGF*	0.7	1.6
157_IL-12B	IL-12B	Interleukin-12 subunit beta	P29460	*IL12B*	1.9	4.1
158_IL-24**	IL-24	Interleukin-24	Q13007	*IL24*	n/a	n/a
159_IL-13**	IL-13	Interleukin-13	P35225	*IL13*	n/a	n/a
160_ARTN**	Artemin	Artemin	Q5T4W7	*ARTN*	n/a	n/a
161_MMP-10	MMP-10	Matrix metalloproteinase-10 (SL-2)	P09238	*MMP10*	0.8	1.7

162_IL-10	IL-10	Interleukin-10	P22301	*IL10*	3.9	5.0
163_TNF**	TNFα	Tumor necrosis factor-alpha	P01375	*TNF*	n/a	n/a
164_CCL23	CCL23	C-C motif chemokine 23	P55773	*CCL23*	0.7	2.7
165_CD5	CD5	T-cell surface glycoprotein CD5	P06127	*CD5*	1.7	2.7
166_MIP-1 alpha	MIP-1α	Macrophage inflammatory protein-1alpha (C-C motif chemokine 3/CCL3)	P10147	*CCL3*	1.6	2.9
167_Flt3L	Flt3L	Fms-related tyrosine kinase 3 ligand	P49771	*FLT3LG*	1.0	2.1
168_CXCL6	CXCL6	C-X-C motif chemokine 6	P80162	*CXCL6*	0.8	2.4
169_CXCL10	CXCL10	C-X-C motif chemokine 10 (IP-10)	P02778	*CXCL10*	0.9	2.9
170_4E-BP1	EIF4EBP1	Eukaryotic translation initiation factor 4E-binding protein 1	Q13541	*EIF4EBP1*	1.4	5.0
171_IL-20**	IL-20	Interleukin-20	Q9NYY1	*IL20*	n/a	n/a
172_SIRT2	SIRT2	SIR2-like protein 2	Q8IXJ6	*SIRT2*	5.7	6.0
173_CCL28	CCL28	C-C motif chemokine 28	Q9NRJ3	*CCL28*	7.4	7.0
174_DNER	DNER	Delta and Notch-like epidermal growth factor-related receptor	Q8NFT8	*DNER*	0.6	1.7
175_EN-RAGE	EN-RAGE	Protein S100-A12 (EN-RAGE)	P80511	*S100A12*	1.9	8.5
176_CD40	CD40	CD40L receptor	P25942	*CD40*	0.8	1.8

177_IL-33**	IL-33	Interleukin-33	O95760	*IL33*	n/a	n/a
178_IFN-gamma**	IFNγ	Interferon-gamma	P01579	*IFNG*	n/a	n/a
179_FGF-19	FGF-19	Fibroblast growth factor 19	O95750	*FGF19*	1.1	3.3
180_IL-4**	IL-4	Interleukin-4	P05112	*IL4*	n/a	n/a
181_LIF**	LIF	Leukemia inhibitory factor	P15018	*LIF*	n/a	n/a
182_NRTN**	Neurturin	Neurturin	Q99748	*NRTN*	n/a	n/a
183_MCP-2	MCP-2	Monocyte chemotactic protein 2 (MCP-2, CCL8)	P80075	*CCL8*	0.7	1.5
184_CASP-8	Caspase-8	Caspase-8	Q14790	*CASP8*	4.8	8.7
185_CCL25	CCL25	C-C motif chemokine 25	O15444	*CCL25*	1.2	2.3
186_CX3CL1	CX3CL1	Fractalkine	P78423	*CX3CL1*	1.9	2.9
187_TNFRSF9	TNFRSF9	Tumor necrosis factor receptor superfamily member 9	Q07011	*TNFRSF9*	1.2	2.0
188_NT-3	NT-3	Neurotrophin-3	P20783	*NTF3*	5.9	7.6
189_TWEAK	TWEAK	Tumor necrosis factor (Ligand) superfamily, member 12 (TWEAK)	O43508	*TNFSF12*	0.8	1.9
190_CCL20	CCL20	C-C motif chemokine 20	P78556	*CCL20*	1.7	3.5
191_ST1A1	ST1A1	Sulfotransferase 1A1	P50225	*SULT1A1*	3.3	5.3

192_STAMPB	STAMBP	STAM-binding protein	O95630	*STAMBP*	2.5	3.8
193_IL-5**	IL-5	Interleukin-5	P05113	*IL5*	n/a	n/a
194_ADA	ADA	Adenosine deaminase	P00813	*ADA*	2.9	4.2
195_TNFB	TNFβ	Tumor necrosis factor-beta (lymphotoxin-alpha/LT-alpha)	P01374	*LTA*	1.8	3.3
196_CSF-1	CSF-1	Macrophage colony-stimulating factor 1	P09603	*CSF1*	0.9	1.7

LOD, limit of detection; n/a, not applicable (NPX of control measurements below LOD); NPX, normalised protein expression values.

*No data reported owing to technical problems with the assay.

**Excluded from analysis because missing data for >25%.

Table S2 - Biomarkers of inflammation in the study population according to diabetes subgroups

Biomarker	Full name	SAID n=87	SIDD n=12	SIRD n=39	MOD n=133	MARD n=143	*P*
IL-8	Interleukin-8	6.74 (6.38; 7.08)	6.68 (6.31; 7.27)	6.99 (6.82; 7.54)	6.87 (6.60; 7.23)	6.98 (6.72; 7.28)	**<0.0001***
VEGF-A	Vascular endothelial growth factor A	10.11 (9.83; 10.54)	9.97 (9.57; 10.53)	10.32 (10.13; 10.81)	10.22 (9.88; 10.6)	10.20 (9.84; 10.61)	0.1335
MCP-3	Monocyte chemotactic protein 3 (CCL7)	2.13 (1.86; 2.58)	2.21 (2.16; 2.70)	2.59 (2.28; 3.37)	2.43 (2.13; 2.74)	2.31 (2.14; 2.61)	**<0.0001***
GDNF	Glial cell line-derived neurotrophic factor	1.71 (1.55; 1.94)	1.69 (1.53; 1.90)	1.90 (1.74; 2.06)	1.81 (1.60; 2.07)	1.78 (1.63; 2.02)	0.082
CDCP1	CUB domain-containing protein 1	2.60 (2.23; 3.07)	3.04 (2.21; 3.31)	3.34 (2.94; 3.69)	3.10 (2.76; 3.52)	3.15 (2.85; 3.54)	**<0.0001***
CD244	Natural killer cell receptor 2B4	5.89 (5.69; 6.09)	5.61 (5.48; 5.81)	5.91 (5.68; 6.13)	5.84 (5.70; 5.99)	5.75 (5.53; 5.91)	**0.0005***
IL-7	Interleukin-7	4.88 (4.59; 5.27)	5.11 (4.62; 5.47)	4.97 (4.60; 5.25)	5.14 (4.75; 5.47)	5.05 (4.72; 5.42)	0.0833
OPG	Osteoprotegerin	10.37 (10.14; 10.56)	10.28 (10.07; 10.51)	10.73 (10.39; 10.95)	10.51 (10.21; 10.82)	10.50 (10.25; 10.74)	**<0.0001***
LAP TGFβ1	Latency-associated peptide transforming growth factor beta-1	8.66 (8.38; 9.00)	8.66 (8.26; 8.92)	8.85 (8.58; 9.11)	8.87 (8.58; 9.09)	8.76 (8.42; 8.95)	**0.0127**
uPA	Urokinase-type plasminogen activator	10.14 (9.90; 10.31)	9.87 (9.76; 10.07)	10.23 (10.03; 10.41)	10.09 (9.91; 10.26)	10.11 (9.87; 10.31)	**0.0039**
IL-6	Interleukin-6	3.15 (2.78; 3.61)	3.51 (2.66; 3.77)	4.20 (3.85; 4.87)	3.90 (3.47; 4.46)	3.65 (3.19; 4.10)	**<0.0001***
IL-17C	Interleukin-17C	1.53 (1.25; 1.88)	1.46 (1.14; 1.51)	1.65 (1.26; 1.96)	1.58 (1.26; 1.90)	1.39 (1.21; 1.74)	**0.0430**
MCP-1	Monocyte chemotactic protein 1 (CCL2)	11.01 (10.76; 11.42)	11.13 (10.80; 11.28)	11.44 (11.12; 11.76)	11.17 (10.90; 11.44)	11.15 (10.89; 11.44)	**0.0061**
IL-17A	Interleukin-17A	1.04 (0.92; 01.2)	0.75 (0.71; 1.09)	1.05 (0.91; 1.28)	0.99 (0.79; 1.28)	0.99 (0.78; 1.20)	0.1924
CXCL11	C-X-C motif chemokine 11	7.78 (7.42; 8.21)	7.48 (7.13; 8.22)	7.96 (7.57; 08.4)	7.78 (7.37; 8.17)	7.80 (7.39; 8.20)	0.5630
Axin-1	Axin-1	1.96 (1.73; 2.28)	1.66 (1.46; 2.11)	1.99 (1.62; 2.46)	1.99 (1.57; 2.33)	1.84 (1.53; 2.10)	**0.0455**
TRAIL	TNF-related apoptosis-inducing ligand (TNFSF10)	8.44 (8.22; 8.63)	8.28 (8.10; 8.76)	8.41 (8.22; 8.73)	8.45 (8.22; 8.63)	8.41 (8.19; 8.63)	0.9023

CXCL9	C-X-C motif chemokine 9	7.13 (6.64; 7.59)	6.91 (6.76; 7.24)	7.33 (6.83; 8.20)	7.02 (6.68; 7.38)	7.34 (6.93; 7.89)	**0.0001***
CST5	Cystatin D	5.81 (5.53; 6.16)	5.68 (5.38; 5.83)	5.94 (5.59; 6.32)	5.62 (5.34; 6.01)	5.88 (5.54; 6.29)	**0.0005***
OSM	Oncostatin-M	4.34 (3.81; 4.94)	4.68 (3.78; 5.02)	4.83 (4.20; 5.35)	4.67 (4.33; 5.24)	4.26 (3.87; 4.77)	**<0.0001***
CXCL1	C-X-C motif chemokine 1	9.03 (8.54; 9.31)	8.76 (8.39; 9.13)	9.02 (8.77; 9.55)	8.98 (8.66; 9.24)	8.90 (8.63; 9.22)	0.1737
CCL4	C-C motif chemokine 4	7.29 (6.84; 7.62)	7.4 (6.77; 7.60)	7.37 (7.04; 7.88)	7.33 (7.02; 7.75)	7.43 (7.03; 7.77)	0.1056
CD6	T cell surface glycoprotein CD6 isoform	4.56 (4.26; 4.81)	4.36 (4.08; 4.44)	4.41 (4.19; 4.70)	4.47 (4.19; 4.73)	4.42 (4.15; 4.71)	0.2836
SCF	Stem cell factor (c-Kit-ligand)	9.91 (9.70; 10.12)	10.00 (9.32; 10.11)	9.58 (9.32; 9.91)	9.58 (9.27; 9.82)	9.81 (9.57; 10.08)	**<0.0001***
IL-18	Interleukin-18	7.84 (7.37; 8.12)	7.88 (7.37; 8.03)	8.17 (7.83; 8.65)	8.02 (7.71; 8.35)	7.86 (7.59; 8.16)	**0.0001***
SLAMF1	Signaling lymphocytic activation molecule (SLAM)	1.83 (1.54; 2.04)	1.81 (1.54; 2.21)	2.06 (1.75; 2.39)	1.89 (1.67; 2.15)	1.83 (1.54; 2.09)	**0.0166**
TGFα	Transforming growth factor alpha	4.08 (3.62; 4.41)	3.93 (3.51; 4.22)	4.2 (3.56; 4.62)	4.16 (3.77; 4.51)	3.94 (3.63; 4.33)	0.0577
MCP-4	Monocyte chemotactic protein 4 (CCL13)	4.53 (4.21; 4.93)	4.84 (3.96; 5.22)	5.09 (4.37; 5.37)	4.63 (4.28; 5.10)	4.80 (4.49; 5.19)	**0.0024**
Eotaxin	Eotaxin (CCL11)	8.02 (7.65; 8.30)	8.10 (7.89; 8.33)	8.24 (7.94; 8.51)	7.98 (7.64; 8.30)	8.21 (8.00; 8.43)	**<0.0001***
TNFSF14	Tumor necrosis factor ligand superfamily member 14 (LIGHT)	5.78 (5.23; 6.22)	5.68 (5.32; 5.81)	5.72 (5.40; 6.36)	5.81 (5.39; 6.18)	5.58 (5.08; 6.03)	**0.0078**
FGF-23	Fibroblast growth factor 23	1.38 (1.22; 1.58)	1.16 (0.92; 1.40)	1.65 (1.25; 1.86)	1.31 (1.09; 1.57)	1.31 (1.13; 1.52)	**0.0006***
IL-10RA	Interleukin-10 receptor subunit alpha	1.06 (0.85; 1.30)	0.90 (0.67; 1.09)	1.09 (0.89; 1.47)	1.04 (0.88; 1.36)	0.98 (0.80; 1.30)	0.1179
FGF-5	Fibroblast growth factor 5	1.07 (0.90; 1.29)	1.10 (1.00; 1.24)	1.12 (1.01; 1.25)	1.06 (0.96; 1.20)	1.10 (0.94; 1.26)	0.5468
MMP-1	Matrix metalloproteinase-1	14.10 (13.38; 14.57)	14.00 (12.98; 14.34)	14.20 (13.65; 14.53)	14.19 (13.53; 14.54)	14.12 (13.41; 14.56)	0.6553
LIF-R	Leukemia inhibitory factor receptor	2.88 (2.72; 3.09)	2.79 (2.67; 3.11)	3.04 (2.76; 3.14)	2.89 (2.74; 3.03)	2.87 (2.71; 3.02)	0.0768
FGF-21	Fibroblast growth factor 21	4.98 (3.95; 6.07)	5.50 (3.75; 6.17)	6.92 (6.40; 8.14)	6.47 (5.52; 7.56)	6.34 (5.61; 7.18)	**<0.0001***
CCL19	C-C motif chemokine 19	9.02 (8.53; 9.48)	8.94 (8.28; 9.15)	9.45 (8.96; 9.96)	9.22 (8.89; 9.68)	8.98 (8.51; 9.51)	**<0.0001***

IL-15RA	Interleukin-15 receptor subunit alpha	0.98 (0.86; 1.09)	0.96 (0.80; 1.09)	1.03 (0.96; 1.23)	0.96 (0.86; 1.07)	0.99 (0.87; 1.12)	0.0565
IL-10RB	Interleukin-10 receptor subunit beta	6.76 (6.60; 6.97)	6.67 (6.42; 6.86)	7.02 (6.77; 7.25)	6.86 (6.68; 7.06)	6.73 (6.53; 6.93)	**<0.0001***
IL-18R1	Interleukin-18 receptor 1	7.05 (6.76; 7.37)	7.46 (6.80; 7.63)	7.32 (7.02; 7.55)	7.31 (7.02; 7.71)	7.07 (6.75; 7.31)	**<0.0001***
PD-L1	Programmed cell death 1 ligand 1	4.03 (3.82; 4.22)	3.77 (3.54; 4.12)	4.02 (3.80; 4.36)	3.93 (3.75; 4.16)	3.90 (3.67; 4.08)	**0.0049**
Beta-NGF	Beta-nerve growth factor	1.79 (1.63; 1.98)	1.66 (1.53; 1.74)	1.86 (1.65; 2.05)	1.77 (1.63; 1.94)	1.70 (1.58; 1.88)	**0.0047**
CXCL5	C-X-C motif chemokine 5	12.02 (11.48; 12.49)	12.06 (11.30; 12.43)	11.94 (11.49; 12.58)	12.11 (11.58; 12.58)	12.06 (11.48; 12.45)	0.8775
TRANCE	TNF-related activation-induced cytokine (TRANCE, TNFSF11, RANKL, OPGL)	4.95 (4.36; 5.48)	4.77 (4.52; 5.17)	4.69 (4.15; 5.07)	4.69 (4.32; 5.13)	4.61 (4.21; 5.09)	**0.0322**
HGF	Hepatocyte growth factor	8.62 (8.36; 8.96)	8.63 (8.32; 9.01)	9.18 (8.86; 9.33)	9.01 (8.74; 9.32)	8.78 (8.55; 9.06)	**<0.0001***
IL-12B	Interleukin-12 subunit beta	4.21 (3.87; 4.75)	3.82 (3.46; 4.13)	4.52 (4.08; 5.20)	4.25 (3.88; 4.78)	4.04 (3.61; 4.39)	**<0.0001***
MMP-10	Matrix metalloproteinase-10 (SL-2)	6.02 (5.61; 6.38)	6.03 (5.59; 6.54)	6.09 (5.79; 6.47)	5.90 (5.59; 6.26)	5.97 (5.63; 6.36)	0.3347
IL-10	Interleukin-10	2.75 (2.45; 3.02)	2.70 (2.58; 2.94)	2.92 (2.68; 3.22)	2.76 (2.50; 3.06)	2.72 (2.46; 3.00)	0.1646
CCL23	C-C motif chemokine 23	9.72 (9.44; 10.09)	9.32 (9.02; 9.67)	9.92 (9.48; 10.17)	9.71 (9.49; 10.04)	9.79 (9.53; 10.08)	**0.0224**
CD5	T-cell surface glycoprotein CD5	4.87 (4.63; 5.10)	4.56 (4.48; 4.71)	5.00 (4.74; 5.22)	4.85 (4.70; 5.01)	4.72 (4.44; 4.93)	**<0.0001***
MIP-1α	Macrophage inflammatory protein-1alpha (C-C motif chemokine 3/CCL3)	5.20 (4.80; 5.57)	5.48 (5.08; 5.67)	5.61 (5.17; 6.05)	5.40 (5.19; 5.71)	5.43 (5.06; 5.74)	**0.0035**
Flt3L	Fms-related tyrosine kinase 3 ligand	8.70 (8.39; 9.02)	8.65 (8.54; 8.92)	9.22 (8.63; 9.38)	8.82 (8.48; 9.13)	8.88 (8.59; 9.18)	**0.0002***
CXCL6	C-X-C motif chemokine 6	8.56 (8.16; 8.96)	8.29 (8.02; 8.71)	8.94 (8.27; 9.41)	8.65 (8.33; 9.01)	8.48 (8.09; 8.87)	**0.0031**
CXCL10	C-X-C motif chemokine 10 (IP-10)	8.24 (7.85; 8.71)	7.85 (7.40; 8.29)	8.57 (8.14; 8.88)	8.38 (7.82; 8.74)	8.23 (7.90; 8.73)	**0.0470**
EIF4EBP1	Eukaryotic translation initiation factor 4E-binding protein 1	7.14 (6.69; 7.66)	7.02 (6.58; 7.68)	7.45 (7.01; 7.85)	7.28 (6.83; 7.85)	7.13 (6.76; 7.62)	0.0889
SIRT2	SIR2-like protein 2	2.09 (1.86; 2.47)	2.1 (1.62; 2.38)	2.29 (1.95; 2.68)	2.21 (1.90; 2.49)	1.99 (1.83; 2.28)	**0.0156**

CCL28	C-C motif chemokine 28	1.42 (1.24; 1.70)	1.24 (0.97; 1.54)	1.44 (1.22; 1.68)	1.33 (1.16; 1.52)	1.46 (1.29; 1.75)	**0.0012**
DNER	Delta and Notch-like epidermal growth factor-related receptor	8.20 (7.98; 8.37)	8.18 (7.98; 8.31)	8.04 (7.90; 8.20)	8.09 (7.86; 8.25)	8.10 (7.92; 8.25)	**0.0217**
EN-RAGE	Protein S100-A12 (EN-RAGE)	5.04 (4.30; 5.74)	4.15 (3.90; 4.80)	5.15 (4.85; 5.68)	5.19 (4.44; 5.62)	4.85 (4.26; 5.46)	**0.0116**
CD40	CD40L receptor	9.73 (9.46; 9.99)	9.57 (9.45; 9.90)	9.93 (9.62; 10.22)	9.80 (9.61; 10.05)	9.77 (9.53; 10.01)	**0.0076**
FGF-19	Fibroblast growth factor 19	8.00 (7.34; 8.57)	8.32 (6.83; 8.93)	8.01 (7.18; 8.53)	7.85 (7.07; 8.43)	8.03 (7.21; 8.60)	0.4946
MCP-2	Monocyte chemotactic protein 2 (MCP-2, CCL8)	8.77 (8.39; 9.18)	8.75 (8.05; 9.21)	8.83 (8.52; 9.33)	8.93 (8.59; 9.39)	8.86 (8.55; 9.33)	0.0887
Caspase-8	Caspase-8	2.08 (1.80; 2.40)	1.68 (1.49; 1.85)	2.27 (1.93; 2.79)	2.16 (1.83; 2.52)	1.99 (1.80; 2.36)	**0.0005***
CCL25	C-C motif chemokine 25	6.22 (5.75; 6.59)	6.44 (6.13; 6.84)	6.48 (6.27; 6.81)	6.22 (5.75; 6.63)	6.34 (6.08; 6.65)	**0.0046**
CX3CL1	Fractalkine	5.80 (5.57; 6.07)	5.68 (5.27; 6.02)	5.73 (5.52; 5.97)	5.64 (5.41; 5.91)	5.69 (5.48; 5.94)	0.0975
TNFRSF9	Tumor necrosis factor receptor superfamily member 9	6.35 (6.06; 6.60)	5.90 (5.62; 6.21)	6.38 (6.14; 6.76)	6.14 (5.95; 6.34)	6.15 (5.87; 6.35)	**<0.0001***
NT-3	Neurotrophin-3	1.65 (1.47; 1.92)	1.38 (1.25; 1.90)	1.70 (1.52; 1.84)	1.61 (1.40; 1.79)	1.58 (1.38; 1.81)	0.1324
TWEAK	Tumor necrosis factor (Ligand) superfamily, member 12 (TWEAK)	9.68 (9.48; 9.84)	9.36 (9.18; 9.62)	9.44 (9.19; 9.60)	9.44 (9.23; 9.67)	9.54 (9.28; 9.74)	**<0.0001***
CCL20	C-C motif chemokine 20	4.83 (4.29; 5.32)	4.53 (4.24; 5.33)	5.47 (4.74 ; 6.86)	5.13 (4.53; 5.73)	4.76 (4.28; 5.35)	**0.0004***
ST1A1	Sulfotransferase 1A1	2.25 (1.73; 2.82)	2.05 (1.58; 2.64)	2.17 (1.80; 3.21)	2.17 (1.69; 2.71)	2.06 (1.68; 2.73)	0.5197
STAMBP	STAM-binding protein	3.66 (3.44; 3.92)	3.62 (3.40; 3.84)	3.78 (3.55; 4.24)	3.76 (3.49; 4.02)	3.60 (3.40; 3.91)	0.0583
ADA	Adenosine deaminase	3.61 (3.41; 3.85)	3.54 (3.19; 3.69)	3.60 (3.40; 3.78)	3.60 (3.38; 3.83)	3.55 (3.32; 3.84)	0.5811
TNFβ	Tumor necrosis factor-beta (lymphotoxin-alpha/LT-alpha)	4.17 (3.89; 4.46)	3.87 (3.68; 4.07)	3.96 (3.71; 4.27)	4.00 (3.71; 4.20)	3.94 (3.66; 4.15)	**<0.0001***
CSF-1	Macrophage colony-stimulating factor 1	7.95 (7.71; 8.11)	7.80 (7.67; 7.97)	8.15 (8.01; 8.28)	8.03 (7.91; 8.18)	7.93 (7.78; 8.06)	**<0.0001***

Data are presented as median (25th percentile; 75th percentile). Differences between diabetes subgroups were analyzed with the Kruskal-Wallis Test.

MARD, moderate age-related diabetes; MOD, moderate obesity-related diabetes; SAID, severe autoimmune diabetes; SIDD, severe insulin-deficient diabetes; SIRD, severe insulin-resistant diabetes.

*Significant Tukey-Kramer *P* values after correction for multiple testing with the Bonferroni method (0.05/74=0.0007).

Table S3 - Unadjusted and adjusted pairwise comparisons of biomarkers of inflammation between diabetes subgroups

Biomarker	Cluster pairs		Model 1 (unadjusted)			Model 2 (adjusted)		
			β	SE	$P_{\text{Tukey-Kramer}}$	β	SE	$P_{\text{Tukey-Kramer}}$
CASP-8	SAID	SIDD	0.4	0.1	**0.00065***	0.6	0.1	**0.0002***
CASP-8	SAID	SIRD	-0.2	0.1	0.2074	-0.1	0.2	0.8812
CASP-8	SAID	MOD	-0.1	0.1	0.7797	-0.02	0.1	0.9994
CASP-8	SAID	MARD	0.02	0.1	0.9959	0.05	0.1	0.9896
CASP-8	SIDD	SIRD	-0.6	0.1	**<0.0001***	-0.7	0.2	**0.0005***
CASP-8	SIDD	MOD	-0.5	0.1	**<0.0001***	-0.6	0.1	**0.0001***
CASP-8	SIDD	MARD	-0.4	0.1	**0.0006***	-0.5	0.1	**0.0005***
CASP-8	SIRD	MOD	0.1	0.1	0.6035	0.1	0.1	0.8514
CASP-8	SIRD	MARD	0.2	0.1	0.0805	0.2	0.1	0.5680
CASP-8	MOD	MARD	0.1	0.1	0.3737	0.07	0.08	0.9199
CCL11	SAID	SIDD	-0.1	0.2	0.9959	0.1	0.1	0.9644
CCL11	SAID	SIRD	-0.3	0.1	**0.0323**	-0.2	0.1	0.5733
CCL11	SAID	MOD	0.01	0.1	0.9989	-0.03	0.09	0.9983
CCL11	SAID	MARD	-0.2	0.1	**0.0044**	-0.09	0.09	0.8280
CCL11	SIDD	SIRD	-0.2	0.2	0.7877	-0.3	0.2	0.4520
CCL11	SIDD	MOD	0.1	0.2	0.9877	-0.1	0.1	0.9150
CCL11	SIDD	MARD	-0.2	0.2	0.8489	-0.2	0.1	0.6891
CCL11	SIRD	MOD	0.3	0.1	**0.0064**	0.2	0.1	0.4433
CCL11	SIRD	MARD	0.03	0.1	0.9933	0.1	0.1	0.8562
CCL11	MOD	MARD	-0.2	0.1	**<0.0001***	-0.07	0.07	0.8875
CCL20	SAID	SIDD	0.2	0.2	0.8355	0.3	0.3	0.8270
CCL20	SAID	SIRD	-0.8	0.2	**0.0042**	-0.7	0.3	0.1862
CCL20	SAID	MOD	-0.3	0.1	0.1121	-0.4	0.2	0.3233

CCL20	SAID	MARD	-0.1	0.1		0.9623			-0.5	0.2	0.1337	
CCL20	SIDD	SIRD	-1	0.3		**0.0039**			-1.0	0.4	0.0789	
CCL20	SIDD	MOD	-0.6	0.2		0.0950			-0.7	0.3	0.1675	
CCL20	SIDD	MARD	-0.3	0.2		0.6058			-0.8	0.3	0.0834	
CCL20	SIRD	MOD	0.5	0.2		0.2606			0.4	0.3	0.6749	
CCL20	SIRD	MARD	0.7	0.2		**0.0203**			0.3	0.3	0.8896	
CCL20	MOD	MARD	0.2	0.1		0.4616			-0.1	0.2	0.9854	
CD5	SAID	SIDD	0.2	0.1		0.1396			0.2	0.1	0.2530	
CD5	SAID	SIRD	-0.1	0.1		0.2334			-0.2	0.1	0.3363	
CD5	SAID	MOD	-0.004	0.04		1.0000			-0.04	0.06	0.9717	
CD5	SAID	MARD	0.1	0.04		0.0678			-0.02	0.07	0.9990	
CD5	SIDD	SIRD	-0.3	0.1		**0.0045**			-0.4	0.1	**0.0148**	
CD5	SIDD	MOD	-0.2	0.1		0.0966			-0.2	0.1	0.0928	
CD5	SIDD	MARD	-0.1	0.1		0.8348			-0.2	0.1	0.1680	
CD5	SIRD	MOD	0.1	0.1		0.1696			0.1	0.08	0.3020	
CD5	SIRD	MARD	0.3	0.1		**0.0002***			0.2	0.08	0.2277	
CD5	MOD	MARD	0.1	0.01		**0.0065**			0.02	0.05	0.9932	
CDCP1	SAID	SIDD	-0.2	0.2		0.8718			0.3	0.1	0.1609	
CDCP1	SAID	SIRD	-0.7	0.1		**<0.0001***			0.03	0.2	0.9997	
CDCP1	SAID	MOD	-0.5	0.1		**<0.0001***			-0.06	0.1	0.9792	
CDCP1	SAID	MARD	-0.6	0.1		**<0.0001***			-0.1	0.1	0.8711	
CDCP1	SIDD	SIRD	-0.5	0.2		0.1380			-0.3	0.2	0.5294	
CDCP1	SIDD	MOD	-0.3	0.2		0.7301			-0.4	0.1	0.0624	
CDCP1	SIDD	MARD	-0.4	0.2		0.4545			-0.4	0.1	**0.0329**	
CDCP1	SIRD	MOD	0.3	0.1		0.1490			-0.09	0.1	0.9632	
CDCP1	SIRD	MARD	0.2	0.1		0.5046			-0.1	0.1	0.8831	
CDCP1	MOD	MARD	-0.1	0.1		0.7367			-0.04	0.09	0.9872	
CSF-1	SAID	SIDD	0.1	0.1		0.3773			0.2	0.09	0.2555	

CSF-1	SAID	SIRD	-0.2	0.04		**<0.0001***			-0.1	0.07	0.2886	
CSF-1	SAID	MOD	-0.1	0.02		**0.0050**			-0.07	0.05	0.6300	
CSF-1	SAID	MARD	0.0001	0.03		1.0000			-0.04	0.05	0.9409	
CSF-1	SIDD	SIRD	-0.4	0.1		**0.0001***			-0.3	0.1	**0.0109**	
CSF-1	SIDD	MOD	-0.3	0.1		**0.0035**			-0.2	0.08	**0.0262**	
CSF-1	SIDD	MARD	-0.1	0.1		0.3252			-0.2	0.08	0.0771	
CSF-1	SIRD	MOD	0.1	0.04		0.2060			0.07	0.05	0.6435	
CSF-1	SIRD	MARD	0.2	0.04		**<0.0001***			0.1	0.06	0.3340	
CSF-1	MOD	MARD	0.1	0.04		**<0.0001***			0.03	0.04	0.9330	
CST5	SAID	SIDD	0.2	0.1		0.6746			0.2	0.1	0.7605	
CST5	SAID	SIRD	-0.1	0.1		0.7797			-0.2	0.15	0.5814	
CST5	SAID	MOD	0.2	0.1		0.0791			0.1	0.09	0.6914	
CST5	SAID	MARD	-0.1	0.1		0.8100			0.005	0.1	1.0000	
CST5	SIDD	SIRD	-0.3	0.2		0.2999			-0.4	0.2	0.2134	
CST5	SIDD	MOD	0	0.1		0.9999			-0.06	0.2	0.9935	
CST5	SIDD	MARD	-0.3	0.1		0.3178			-0.2	0.2	0.7742	
CST5	SIRD	MOD	0.3	0.1		**0.0294**			0.3	0.1	**0.0316**	
CST5	SIRD	MARD	0.04	0.1		0.9902			0.2	0.1	0.3721	
CST5	MOD	MARD	-0.2	0.1		**0.0002***			-0.1	0.08	0.5909	
CXCL9	SAID	SIDD	0.3	0.2		0.6490			0.4	0.2	0.5931	
CXCL9	SAID	SIRD	-0.3	0.2		0.4362			-0.2	0.2	0.8664	
CXCL9	SAID	MOD	0.2	0.1		0.3794			0.1	0.1	0.8937	
CXCL9	SAID	MARD	-0.2	0.1		0.4334			0.05	0.2	0.9974	
CXCL9	SIDD	SIRD	-0.6	0.3		0.1453			-0.6	0.3	0.2683	
CXCL9	SIDD	MOD	-0.1	0.2		0.9685			-0.2	0.2	0.8653	
CXCL9	SIDD	MARD	-0.5	0.2		0.1789			-0.3	0.2	0.6981	
CXCL9	SIRD	MOD	0.5	0.1		**0.0147**			0.3	0.2	0.2333	
CXCL9	SIRD	MARD	0.1	0.1		0.9756			0.3	0.2	0.5680	
CXCL9	MOD	MARD	-0.4	0.1		**0.0001***			-0.08	0.1	0.9556	

EN-RAGE	SAID	SIDD	0.6	0.2		**0.0143**			0.7	0.2	**0.0143**	
EN-RAGE	SAID	SIRD	-0.1	0.2		0.9417			-0.3	0.3	0.7567	
EN-RAGE	SAID	MOD	0.1	0.1		0.9982			-0.2	0.1	0.8716	
EN-RAGE	SAID	MARD	0.2	0.1		0.6270			-0.1	0.2	0.9458	
EN-RAGE	SIDD	SIRD	-0.8	0.2		**0.0067**			-1.0	0.3	**0.0049**	
EN-RAGE	SIDD	MOD	-0.7	0.2		**0.0044**			-0.9	0.2	**0.0005***	
EN-RAGE	SIDD	MARD	-0.5	0.2		0.0946			-0.8	0.2	**0.0010**	
EN-RAGE	SIRD	MOD	0.1	0.2		0.9777			0.1	0.2	0.9497	
EN-RAGE	SIRD	MARD	0.3	0.2		0.3242			0.2	0.2	0.9074	
EN-RAGE	MOD	MARD	0.2	0.1		0.2648			0.04	0.1	0.9990	
FGF-21	SAID	SIDD	-0.1	0.5		0.9999			0.9	0.6	0.5317	
FGF-21	SAID	SIRD	-2.1	0.3		**<0.0001**			-0.09	0.4	0.9996	
FGF-21	SAID	MOD	-1.4	0.2		**<0.0001***			-0.2	0.3	0.9235	
FGF-21	SAID	MARD	-1.4	0.2		**<0.0001***			-0.3	0.3	0.8145	
FGF-21	SIDD	SIRD	-2.1	0.5		**0.0006***			-0.9	0.6	0.5569	
FGF-21	SIDD	MOD	-1.3	0.5		0.0614			-1.1	0.6	0.2777	
FGF-21	SIDD	MARD	-1.3	0.5		0.0535			-1.2	0.6	0.2160	
FGF-21	SIRD	MOD	0.8	0.2		**0.0116**			-0.1	0.3	0.9915	
FGF-21	SIRD	MARD	0.7	0.2		**0.0073**			-0.2	0.3	0.9613	
FGF-21	MOD	MARD	-0.009	0.2		1.0000			-0.08	0.2	0.9966	
FGF-23	SAID	SIDD	0.2	0.1		0.1238			0.2	0.1	0.4951	
FGF-23	SAID	SIRD	-0.3	0.1		**0.0297**			-0.1	0.1	0.8394	
FGF-23	SAID	MOD	0.02	0.1		0.9898			0.09	0.07	0.7488	
FGF-23	SAID	MARD	0.1	0.04		0.7723			0.06	0.07	0.8984	
FGF-23	SIDD	SIRD	-0.5	0.1		**0.0006***			-0.3	0.2	0.2820	
FGF-23	SIDD	MOD	-0.2	0.1		0.2241			-0.09	0.1	0.9368	
FGF-23	SIDD	MARD	-0.2	0.1		0.3612			-0.1	0.1	0.8557	
FGF-23	SIRD	MOD	0.3	0.1		**0.0147**			0.2	0.1	0.2944	

FGF-23	SIRD	MARD	0.3	0.1		**0.0040**			0.2	0.1	0.4405	
FGF-23	MOD	MARD	0.03	0.04		0.9707			-0.02	0.07	0.9965	
Flt3L	SAID	SIDD	-0.01	0.2		1			0.2	0.1	0.5266	
Flt3L	SAID	SIRD	-0.4	0.1		**0.0005***			-0.01	0.1	1.0000	
Flt3L	SAID	MOD	-0.1	0.1		0.2505			0.03	0.09	0.9972	
Flt3L	SAID	MARD	-0.2	0.06		**0.0026**			0.06	0.09	0.9671	
Flt3L	SIDD	SIRD	-0.4	0.2		0.1148			-0.2	0.2	0.6684	
Flt3L	SIDD	MOD	-0.1	0.1		0.9021			-0.2	0.1	0.6387	
Flt3L	SIDD	MARD	-0.2	0.1		0.5005			-0.2	0.1	0.7695	
Flt3L	SIRD	MOD	0.3	0.1		**0.0354**			0.04	0.1	0.9954	
Flt3L	SIRD	MARD	0.2	0.1		0.3761			0.07	0.1	0.9714	
Flt3L	MOD	MARD	-0.1	0.1		0.3359			0.03	0.08	0.9947	
HGF	SAID	SIDD	0.1	0.1		0.9941			0.2	0.1	0.4593	
HGF	SAID	SIRD	-0.5	0.1		**<0.0001***			0.03	0.1	0.9996	
HGF	SAID	MOD	-0.4	0.1		**<0.0001***			-0.06	0.08	0.9261	
HGF	SAID	MARD	-0.1	0.1		0.1169			-0.07	0.08	0.9130	
HGF	SIDD	SIRD	-0.5	0.1		**0.0011**			-0.2	0.2	0.7373	
HGF	SIDD	MOD	-0.4	0.1		**0.0090**			-0.3	0.1	0.1839	
HGF	SIDD	MARD	-0.2	0.1		0.5284			-0.3	0.1	0.1727	
HGF	SIRD	MOD	0.1	0.1		0.5542			-0.09	0.09	0.8625	
HGF	SIRD	MARD	0.3	0.1		**<0.0001***			-0.09	0.1	0.8627	
HGF	MOD	MARD	0.2	0.04		**<0.0001***			0.003	0.07	1.0000	
IL-6	SAID	SIDD	0.04	0.2		0.9998			0.6	0.2	**0.0181**	
IL-6	SAID	SIRD	-1.1	0.1		**<0.0001***			-0.4	0.2	0.2805	
IL-6	SAID	MOD	-0.7	0.1		**<0.0001***			-0.2	0.1	0.5610	
IL-6	SAID	MARD	-0.5	0.1		**<0.0001***			-0.3	0.1	0.1295	
IL-6	SIDD	SIRD	-1.1	0.2		**<0.0001***			-0.9	0.2	**0.0008**	
IL-6	SIDD	MOD	-0.7	0.2		**0.0140**			-0.8	0.2	**0.0008**	

IL-6	SIDD	MARD	-0.5	0.2		0.1696			-0.9	0.2	**0.0001***	
IL-6	SIRD	MOD	0.4	0.1		**0.0097**			0.2	0.1	0.6858	
IL-6	SIRD	MARD	0.6	0.1		**<0.0001***			0.09	0.1	0.9814	
IL-6	MOD	MARD	0.2	0.1		0.116			-0.1	0.1	0.8598	
IL_10RB	SAID	SIDD	0.1	0.1		0.5045			0.2	0.1	0.3976	
IL_10RB	SAID	SIRD	-0.2	0.1		**0.0002***			-0.04	0.09	0.9893	
IL_10RB	SAID	MOD	-0.1	0.04		0.247			0.01	0.06	0.9997	
IL_10RB	SAID	MARD	0.02	0.03		0.9796			0.02	0.06	0.9987	
IL_10RB	SIDD	SIRD	-0.4	0.1		**0.0006***			-0.2	0.1	0.3677	
IL_10RB	SIDD	MOD	-0.2	0.1		0.0738			-0.2	0.1	0.4603	
IL_10RB	SIDD	MARD	-0.1	0.1		0.6401			-0.2	0.1	0.4945	
IL_10RB	SIRD	MOD	0.2	0.1		**0.0266**			0.05	0.07	0.9376	
IL_10RB	SIRD	MARD	0.3	0.1		**<0.0001***			0.06	0.07	0.9276	
IL_10RB	MOD	MARD	0.1	0.02		**0.0229**			0.005	0.05	1.0000	
IL_12B	SAID	SIDD	0.5	0.1		**0.0055**			0.4	0.2	0.1687	
IL_12B	SAID	SIRD	-0.3	0.1		0.2206			-0.3	0.2	0.4858	
IL_12B	SAID	MOD	0.009	0.1		1.0000			0.06	0.1	0.9886	
IL_12B	SAID	MARD	0.3	0.1		**0.0181**			0.2	0.1	0.6559	
IL_12B	SIDD	SIRD	-0.8	0.2		**<0.0001***			-0.7	0.2	**0.0212**	
IL_12B	SIDD	MOD	-0.5	0.1		**0.0047**			-0.3	0.2	0.3056	
IL_12B	SIDD	MARD	-0.3	0.1		0.3611			-0.2	0.2	0.6996	
IL_12B	SIRD	MOD	0.3	0.1		0.1610			0.4	0.2	0.1241	
IL_12B	SIRD	MARD	0.5	0.1		**0.0005***			0.5	0.2	**0.0323**	
IL_12B	MOD	MARD	0.2	0.1		**0.0084**			0.1	0.1	0.8350	
IL_17C	SAID	SIDD	0.2	0.1		0.1642			0.3	0.1	0.0624	
IL_17C	SAID	SIRD	-0.1	0.1		0.8673			-0.2	0.2	0.8481	
IL_17C	SAID	MOD	-0.04	0.1		0.9575			-0.05	0.1	0.9903	
IL_17C	SAID	MARD	0.04	0.1		0.975			-0.2	0.1	0.4189	

IL_17C	SIDD	SIRD	-0.3	0.1		**0.0488**			-0.4	0.2	0.0546	
IL_17C	SIDD	MOD	-0.3	0.1		**0.0387**			-0.3	0.1	**0.0285**	
IL_17C	SIDD	MARD	-0.2	0.1		0.4123			-0.5	0.1	**0.0008**	
IL_17C	SIRD	MOD	0.04	0.1		0.9915			0.1	0.1	0.8957	
IL_17C	SIRD	MARD	0.1	0.1		0.6042			-0.02	0.1	0.9999	
IL_17C	MOD	MARD	0.1	0.1		0.6940			-0.1	0.1	0.6421	
IL_18R1	SAID	SIDD	-0.2	0.2		0.8690			0.06	0.2	0.9977	
IL_18R1	SAID	SIRD	-0.3	0.1		**0.0117**			-0.1	0.1	0.7529	
IL_18R1	SAID	MOD	-0.3	0.1		**<0.0001***			-0.2	0.08	0.0907	
IL_18R1	SAID	MARD	0.01	0.1		0.9980			-0.08	0.08	0.8667	
IL_18R1	SIDD	SIRD	-0.1	0.2		0.9950			-0.2	0.2	0.8392	
IL_18R1	SIDD	MOD	-0.1	0.2		0.9879			-0.3	0.2	0.5793	
IL_18R1	SIDD	MARD	0.2	0.2		0.8163			-0.1	0.2	0.9368	
IL_18R1	SIRD	MOD	-0.01	0.1		0.9995			-0.06	0.1	0.9796	
IL_18R1	SIRD	MARD	0.3	0.1		**0.0017**			0.07	0.1	0.9583	
IL_18R1	MOD	MARD	0.3	0.1		**<0.0001***			0.1	0.07	0.3791	
MCP_3	SAID	SIDD	-0.1	0.1		0.9104			0.07	0.1	0.9864	
MCP_3	SAID	SIRD	-0.5	0.1		**<0.0001***			-0.1	0.2	0.8723	
MCP_3	SAID	MOD	-0.3	0.1		**0.0058**			-0.07	0.1	0.9539	
MCP_3	SAID	MARD	-0.2	0.1		0.1182			-0.1	0.1	0.8608	
MCP_3	SIDD	SIRD	-0.4	0.2		0.1400			-0.2	0.2	0.7216	
MCP_3	SIDD	MOD	-0.1	0.1		0.8605			-0.1	0.1	0.8072	
MCP_3	SIDD	MARD	-0.04	0.1		0.9975			-0.2	0.1	0.6727	
MCP_3	SIRD	MOD	0.2	0.1		0.1602			0.08	0.1	0.9671	
MCP_3	SIRD	MARD	0.3	0.1		**0.0097**			0.05	0.1	0.9940	
MCP_3	MOD	MARD	0.1	0.1		0.5319			-0.03	0.08	0.9974	
OPG	SAID	SIDD	0.1	0.1		0.9558			0.3	0.1	0.0790	
OPG	SAID	SIRD	-0.4	0.1		**<0.0001***			-0.06	0.1	0.9908	

OPG	SAID	MOD	-0.2	0.1		**0.0125**			-0.02	0.08	0.9984	
OPG	SAID	MARD	-0.2	0.1		**0.0123**			0.08	0.08	0.8283	
OPG	SIDD	SIRD	-0.5	0.1		**0.0053**			-0.4	0.1	0.1243	
OPG	SIDD	MOD	-0.3	0.1		0.165			-0.3	0.1	**0.0469**	
OPG	SIDD	MARD	-0.3	0.1		0.1925			-0.2	0.1	0.3294	
OPG	SIRD	MOD	0.2	0.1		0.1159			0.03	0.1	0.9967	
OPG	SIRD	MARD	0.2	0.1		0.0624			0.1	0.1	0.6546	
OPG	MOD	MARD	0.01	0.04		0.9989			0.1	0.07	0.5158	
OSM	SAID	SIDD	-0.1	0.2		0.9995			0.3	0.2	0.7437	
OSM	SAID	SIRD	-0.4	0.2		0.0777			-0.3	0.2	0.7604	
OSM	SAID	MOD	-0.4	0.1		**0.0076**			-0.1	0.1	0.8439	
OSM	SAID	MARD	0.1	0.1		0.8790			-0.1	0.1	0.8982	
OSM	SIDD	SIRD	-0.4	0.3		0.6510			-0.6	0.3	0.2945	
OSM	SIDD	MOD	-0.3	0.2		0.6804			-0.4	0.2	0.3070	
OSM	SIDD	MARD	0.1	0.2		0.9689			-0.4	0.2	0.3513	
OSM	SIRD	MOD	0.05	0.1		0.9975			0.1	0.2	0.9593	
OSM	SIRD	MARD	0.5	0.1		**0.0052**			0.1	0.2	0.9458	
OSM	MOD	MARD	0.5	0.1		**<0.0001***			0.02	0.1	0.9999	
SCF	SAID	SIDD	0.1	0.1		0.8974			-0.1	0.1	0.9482	
SCF	SAID	SIRD	0.3	0.1		**0.0032**			-0.2	0.1	0.5579	
SCF	SAID	MOD	0.4	0.1		**<0.0001***			0.1	0.07	0.4192	
SCF	SAID	MARD	0.1	0.04		0.1193			0.04	0.07	0.9678	
SCF	SIDD	SIRD	0.1	0.1		0.8548			-0.09	0.1	0.9827	
SCF	SIDD	MOD	0.2	0.1		0.3394			0.2	0.1	0.4614	
SCF	SIDD	MARD	-0.004	0.1		1.0000			0.1	0.1	0.8302	
SCF	SIRD	MOD	0.1	0.1		0.6342			0.3	0.1	**0.0187**	
SCF	SIRD	MARD	-0.1	0.1		0.2559			0.2	0.1	0.1811	
SCF	MOD	MARD	-0.3	0.1		**<0.0001***			-0.08	0.07	0.7821	

TNFB	SAID	SIDD	0.3	0.1		**0.0157**			0.2	0.1	0.4563	
TNFB	SAID	SIRD	0.2	0.1		0.1536			0.1	0.1	0.7571	
TNFB	SAID	MOD	0.2	0.1		**0.0078**			0.1	0.08	0.3918	
TNFB	SAID	MARD	0.3	0.1		**<0.0001***			0.1	0.08	0.3659	
TNFB	SIDD	SIRD	-0.1	0.1		0.9086			-0.02	0.1	0.9999	
TNFB	SIDD	MOD	-0.1	0.1		0.7033			-0.04	0.1	0.9964	
TNFB	SIDD	MARD	-0.033	0.1		0.9959			-0.03	0.1	0.9986	
TNFB	SIRD	MOD	-0.01	0.1		0.9996			-0.02	0.1	0.9998	
TNFB	SIRD	MARD	0.1	0.1		0.9336			-0.01	0.1	1.0000	
TNFB	MOD	MARD	0.1	0.04		0.3621			0.01	0.06	1.0000	
TNFRSF9	SAID	SIDD	0.4	0.1		**0.0018**			0.4	0.1	**0.0142**	
TNFRSF9	SAID	SIRD	-0.1	0.1		0.5317			-0.1	0.1	0.8280	
TNFRSF9	SAID	MOD	0.2	0.1		**0.0307**			0.1	0.08	0.3989	
TNFRSF9	SAID	MARD	0.2	0.1		**0.0260**			0.1	0.08	0.4157	
TNFRSF9	SIDD	SIRD	-0.5	0.1		**<0.0001***			-0.5	0.1	**0.0054**	
TNFRSF9	SIDD	MOD	-0.2	0.1		0.1405			-0.2	0.1	0.2240	
TNFRSF9	SIDD	MARD	-0.2	0.1		0.1803			-0.2	0.1	0.2599	
TNFRSF9	SIRD	MOD	0.3	0.1		**0.0004***			0.3	0.09	**0.0261**	
TNFRSF9	SIRD	MARD	0.3	0.1		**0.0004***			0.3	0.09	**0.0385**	
TNFRSF9	MOD	MARD	0.008	0.04		0.9997			0.005	0.06	1.0000	
TWEAK	SAID	SIDD	0.3	0.1		**0.0241**			0.1	0.1	0.4930	
TWEAK	SAID	SIRD	0.2	0.1		**0.0012**			-0.2	0.1	0.4189	
TWEAK	SAID	MOD	0.2	0.04		**<0.0001***			0.004	0.06	1.0000	
TWEAK	SAID	MARD	0.2	0.04		**0.0022**			-0.02	0.06	0.9980	
TWEAK	SIDD	SIRD	-0.1	0.1		0.9821			-0.3	0.1	0.0669	
TWEAK	SIDD	MOD	-0.1	0.1		0.9387			-0.1	0.1	0.5328	
TWEAK	SIDD	MARD	-0.1	0.1		0.6215			-0.2	0.1	0.3850	
TWEAK	SIRD	MOD	-0.01	0.1		0.9986			0.2	0.08	0.1648	
TWEAK	SIRD	MARD	-0.1	0.1		0.6701			0.1	0.08	0.3439	

TWEAK	MOD	MARD	-0.06	0.04		0.5787			-0.02	0.05	0.9930	
uPA	SAID	SIDD	0.2	0.1		**0.0091**			0.2	0.1	**0.0288**	
uPA	SAID	SIRD	-0.1	0.1		0.1524			0.005	0.1	1.0000	
uPA	SAID	MOD	0.03	0.04		0.976			0.07	0.06	0.7939	
uPA	SAID	MARD	0.01	0.04		0.9989			0.04	0.06	0.9621	
uPA	SIDD	SIRD	-0.4	0.1		**<0.0001***			-0.25	0.1	0.1375	
uPA	SIDD	MOD	-0.2	0.1		**0.0167**			-0.2	0.08	0.1754	
uPA	SIDD	MARD	-0.2	0.1		**0.0069**			-0.2	0.08	0.0876	
uPA	SIRD	MOD	0.2	0.1		**0.0211**			0.06	0.07	0.8728	
uPA	SIRD	MARD	0.1	0.04		**0.0424**			0.04	0.07	0.9837	
uPA	MOD	MARD	-0.01	0.03		0.9939			-0.03	0.05	0.9836	

Only biomarkers with at least one significant pairwise comparison are reported.

Model 2: adjustment for age at diagnosis, BMI, HbA1c, HOMA2-IR, HOMA2-B and glutamic acid decarboxylase antibodies.

Beta coefficients estimate the mean difference between cluster 1 and cluster 2 within each cluster pair (in NPX as biomarker unit).

Bold print indicates significant differences at $P<0.05$ after correction for pairwise multiple comparisons using the Tukey-Kramer test.

* indicates significant differences at $P<0.0007$ after additional Bonferroni correction (0.05/74=0.0007).

CASP-8, caspase-8; CCL11, eotaxin; CCL20/C-C motif chemokine 20; CD5, T-cell surface glycoprotein CD5; CDCP1, CUB domain-containing protein 1; CSF-1, macrophage colony-stimulating factor 1; CST5, cystatin D; CXCL9, C-X-C motif chemokine 9; EN-RAGE, protein S100-A12; FGF21, fibroblast growth factor 21; FGF-23, fibroblast growth factor 23; Flt3L, Fms-related tyrosine kinase 3 ligand; HGF, hepatocyte growth factor; IL-6, interleukin-6; IL-10RB, interleukin-10 receptor subunit beta; IL-12B, interleukin-12 subunit beta; IL-17C, interleukin-17C; IL-18R1,

interleukin-18 receptor 1; MARD, mild age-related diabetes ; MCP-3, monocyte chemotactic protein 3; MOD, mild obesity-related diabetes; OPG, osteoprotegerin; OSM, oncostatin-M; SAID, severe autoimmune diabetes; SCF, stem cell factor; SE, standard error; SIDD, severe insulin-deficient diabetes; SIRD, severe insulin resistant diabetes; TNFB, tumor necrosis factor-beta; TNFRSF9, tumor necrosis factor receptor superfamily member 9; TWEAK, tumor necrosis factor (Ligand) superfamily, member 12; uPA, urokinase-type plasminogen activator.

Table S4 - Spearman correlations between biomarkers of inflammation and variables used in the definition of diabetes subgroups

	Age		BMI		HbA1c		HOMA2-B		HOMA2-IR		GADA	
Biomarker	***r***	***P***	***r***	***P***	***r***	***P***	***r***	***P***	***r***	***P***	***r***	***P***
CASP-8	0.004	0.941	**0.119**	**0.016**	0.032	0.515	**0.154**	**0.002**	0.073	0.135	-0.039	0.434
CCL11	**0.310**	**<0.0001**	0.035	0.475	0.040	0.411	0.060	0.221	0.044	0.371	-0.089	0.067
CCL20	-0.045	0.353	**0.201**	**<0.0001**	**0.111**	**0.022**	**0.160**	**0.001**	**0.211**	**<0.0001**	-0.060	0.219
CD5	**-0.169**	**0.001**	0.041	0.406	0.023	0.635	0.092	0.063	0.071	0.152	0.006	0.899
CDCP1	**0.504**	**<0.0001**	**0.399**	**<0.0001**	**0.136**	**0.006**	**0.201**	**<0.0001**	**0.419**	**<0.0001**	**-0.331**	**<0.0001**
CSF-1	-0.029	0.558	**0.295**	**<0.0001**	0.035	0.479	**0.214**	**<0.0001**	**0.274**	**<0.0001**	**-0.124**	**0.011**
CST5	**0.145**	**0.003**	-0.096	0.051	-0.020	0.69	-0.037	0.455	-0.059	0.234	0.032	0.514
CXCL9	**0.340**	**<0.0001**	0.055	0.267	-0.038	0.441	**0.098**	**0.047**	0.028	0.575	-0.034	0.488
EN-RAGE	**-0.100**	**0.041**	0.069	0.157	0.010	0.839	0.073	0.141	0.017	0.731	0.014	0.762
FGF-21	**0.354**	**<0.0001**	**0.419**	**<0.0001**	0.038	0.446	**0.289**	**<0.0001**	**0.485**	**<0.0001**	**-0.354**	**<0.0001**
FGF-23	0.004	0.929	0.078	0.115	-0.062	0.206	**0.183**	**0.0002**	0.109	**0.027**	0.010	0.839
Flt3L	**0.390**	**<0.0001**	**0.223**	**<0.0001**	0.081	0.100	**0.133**	**0.007**	**0.284**	**<0.0001**	**-0.165**	**0.001**
HGF	**0.110**	**0.025**	**0.461**	**<0.0001**	0.070	0.157	**0.329**	**<0.0001**	**0.467**	**<0.0001**	**-0.235**	**<0.0001**
IL-6	**0.260**	**<0.0001**	**0.544**	**<0.0001**	**0.133**	**0.007**	**0.353**	**<0.0001**	**0.480**	**<0.0001**	**-0.319**	**<0.0001**
IL-10RB	0.017	0.736	**0.230**	**<0.0001**	0.082	0.096	**0.172**	**0.0004**	**0.313**	**<0.0001**	**-0.100**	**0.042**
IL-12B	-0.061	0.215	**0.151**	**0.002**	-0.025	0.611	**0.115**	**0.020**	**0.101**	**0.039**	0.047	0.339
IL-17C	**-0.138**	**0.004**	**0.100**	**0.041**	0.090	0.064	0.078	0.113	0.073	0.137	0.038	0.439
IL-18R1	-0.029	0.559	**0.285**	**<0.0001**	**0.250**	**<0.0001**	0.030	0.539	**0.332**	**<0.0001**	**-0.144**	**0.003**
MCP-3	**0.191**	**<0.0001**	**0.356**	**<0.0001**	0.065	0.184	**0.228**	**<0.0001**	**0.325**	**<0.0001**	**-0.223**	**<0.0001**
OPG	**0.340**	**<0.0001**	**0.253**	**<0.0001**	0.080	0.104	**0.186**	**0.0001**	**0.281**	**<0.0001**	**-0.208**	**<0.0001**
OSM	**-0.126**	**0.010**	**0.251**	**<0.0001**	**0.173**	**<0.0001**	**0.101**	**0.040**	**0.207**	**<0.0001**	-0.093	0.057
SCF	-0.009	0.856	**-0.333**	**<0.0001**	**-0.145**	**0.003**	**-0.171**	**0.0005**	**-0.370**	**<0.0001**	**0.180**	**<0.0001**
TNFB	**-0.217**	**<0.0001**	**-0.119**	**0.016**	-0.034	0.495	-0.045	0.366	**-0.132**	**0.007**	**0.197**	**<0.0001**
TNFRSF9	0.011	0.831	0.012	0.805	-0.013	0.799	**0.098**	**0.046**	-0.001	0.981	**0.110**	**0.025**
TWEAK	**-0.153**	**0.002**	**-0.266**	**<0.0001**	-0.040	0.420	**-0.137**	**0.005**	**-0.344**	**<0.0001**	**0.199**	**<0.0001**
uPA	**0.136**	**0.006**	**0.110**	**0.026**	-0.026	0.593	**0.141**	**0.004**	**0.125**	**0.011**	-0.029	0.55

The table shows only the 26 biomarkers of inflammation with at least one significant pairwise cluster difference after adjustment for multiple testing with both Tukey-Kramer and Bonferroni corrections in either the unadjusted or in the adjusted model.

BMI, body mass index; CASP-8, caspase-8; CCL11, eotaxin; CCL20/C-C motif chemokine 20; CD5, T-cell surface glycoprotein CD5; CDCP1, CUB domain-containing protein 1; CSF-1, macrophage colony-stimulating factor 1; CST5, cystatin D; CXCL9, C-X-C motif chemokine 9; EN-RAGE, protein S100-A12; FGF21, fibroblast growth factor 21; FGF-23, fibroblast growth factor 23; Flt3L, Fms-related tyrosine kinase 3 ligand; GADA, glutamic acid decarboxylase antibodies; HGF, hepatocyte growth factor; HOMA-B, homeostasis model assessment of β-cell function; HOMA-IR, homeostasis model assessment of insulin resistance. IL-6, interleukin-6; IL-10RB, interleukin-10 receptor subunit beta; IL-12B, interleukin-12 subunit beta; IL-17C, interleukin-17C; IL-18R1, interleukin-18 receptor 1; MCP-3, monocyte chemotactic protein 3; OPG, osteoprotegerin; OSM, oncostatin-M; SCF, stem cell factor; TNFB, tumor necrosis factor-beta; TNFRSF9, tumor necrosis factor receptor superfamily member 9; TWEAK, tumor necrosis factor (ligand) superfamily, member 12; uPA, urokinase-type plasminogen activator.

Table S5 - Mendelian randomization results based on EN-RAGE (S100-A12) as exposure and diabetes and diabetes-related complications as outcomes

Outcome	IVW β (SE)	IVW *P* value	IV (SNP)
Type 2 diabetes	0.030 (0.027)	0.276	rs3014874 rs62143206
Type 2 diabetes with diabetic angiopathy	0.240 (0.216)	0.267	rs3014874 rs62143206
Type 2 diabetes with renal complications	0.279 (0.131)	0.033	rs3014874 rs62143206
Type 2 diabetes with diabetic retinopathy	-0.010 (0.091)	0.905	rs3014874 rs62143206
Type 2 diabetes with diabetic neuropathy	0.150 (0.148)	0.310	rs3014874 rs62143206
Type 1 diabetes	0.054 (0.058)	0.353	rs3014874 rs62143206
Type 1 diabetes with diabetic angiopathy	-0.073 (0.287)	0.797	rs3014874 rs62143206
Type 1 diabetes with renal complications	0.151 (0.159)	0.343	rs3014874 rs62143206
Type 1 diabetes with diabetic retinopathy	0.019 (0.112)	0.865	rs3014874 rs62143206
Type 1 diabetes with diabetic neuropathy	-0.136 (0.266)	0.607	rs62143206 rs62143206

IV, instrumental variable; IVW, inverse variance weighted random effects model; SE, standard error; SNP, single nucleotide polymorphism.

Definition of the diabetic complications in the FinnGen Biobank (https://risteys.finngen.fi/):
-Diabetic angiopathy: peripheral circulatory complications; vascular diseases that are associated with diabetes mellitus.
-Renal complications: no further definition available.

-Diabetic retinopathy: ophthalmic complications; a chronic, pathological complication associated with diabetes mellitus, where retinal damages are incurred due to microaneurysms in the vasculature of the retina, progressively leading to abnormal blood vessel growth, and swelling and leaking of fluid from blood vessels, resulting in vision loss or blindness.

-Diabetic neuropathy: neurological complications; a chronic, pathological complication associated with diabetes mellitus, where nerve damages are incurred due to diabetic microvascular injury involving small blood vessels that supply these nerves, resulting in peripheral and/or autonomic nerve dysfunction.

Table S6 - Mendelian randomization results based on IL-6 as exposure and diabetes and diabetes-related complications as outcomes

Outcome	IVW β (SE)	IVW *P* value	IV (SNP)
Type 2 diabetes	0.097(0.089)	0.273	rs11872808 rs75892156
Type 2 diabetes with diabetic angiopathy	0.776 (0.810)	0.338	rs11872808 rs75892156
Type 2 diabetes with renal complications	0.330 (0.383)	0.388	rs11872808 rs75892156
Type 2 diabetes with diabetic retinopathy	0.533 (0.311)	0.087	rs11872808 rs75892156
Type 2 diabetes with diabetic neuropathy	0.544 (0.405)	0.179	rs11872808 rs75892156
Type 1 diabetes	0.076 (0.161)	0.633	rs11872808 rs75892156
Type 1 diabetes with diabetic angiopathy	-0.222 (0.633)	0.725	rs11872808 rs75892156
Type 1 diabetes with renal complications	-0.269 (0.415)	0.516	rs11872808 rs75892156
Type 1 diabetes with diabetic retinopathy	0.170 (0.261)	0.513	rs11872808 rs75892156
Type 1 diabetes with diabetic neuropathy	0.172 (0.485)	0.722	rs11872808 rs75892156

IV, instrumental variable ; IVW, inverse variance weighted random effects model; SE, standard error; SNP, single nucleotide polymorphism.

Definition of the diabetic complications in the FinnGen Biobank (https://risteys.finngen.fi/):
-Diabetic angiopathy: peripheral circulatory complications; vascular diseases that are associated with diabetes mellitus.
-Renal complications: no further definition available.
-Diabetic retinopathy: ophthalmic complications; a chronic, pathological complication associated with diabetes mellitus, where retinal damages are incurred due to microaneurysms

in the vasculature of the retina, progressively leading to abnormal blood vessel growth, and swelling and leaking of fluid from blood vessels, resulting in vision loss or blindness.

-Diabetic neuropathy: neurological complications; a chronic, pathological complication associated with diabetes mellitus, where nerve damages are incurred due to diabetic microvascular injury involving small blood vessels that supply these nerves, resulting in peripheral and/or autonomic nerve dysfunction.

Table S7 - Mendelian randomization results based on diabetes and diabetes-related complications as exposures and EN-RAGE (S100-A12) as outcome

Exposure	Method	β (SE)	*P*	Number of SNPs (IV)	Pleiotropy test *
Type 2 diabetes	IVW	0.036 (0.089)	0.684	7	0.727
Type 2 diabetes with diabetic retinopathy	Wald	0.014 (0.090)	0.870	1	NA
Type 1 diabetes	IVW	0.001 (0.033)	0.972	7	0.703
Type 1 diabetes with diabetic angiopathy	Wald	-0.002 (0.026)	0.916	1	NA
Type 1 diabetes with renal complications	IVW	0.010 (0.021)	0.630	2	NA
Type 1 diabetes with diabetic retinopathy	IVW	-0.004 (0.030)	0.886	5	0.944
Type 1 diabetes with diabetic neuropathy	IVW	-0.015 (0.011)	0.161	4	0.947

* Pleiotropy *P* value represents the *P* value of the intercept of Egger's regression.
IV, instrumental variable; IVW, inverse variance weighted random effects model; NA, not applicable; SE, standard error; SNP, single nucleotide polymorphism.

Definition of the diabetic complications in the FinnGen Biobank (https://risteys.finngen.fi/):
-Diabetic angiopathy: peripheral circulatory complications; vascular diseases that are associated with diabetes mellitus.
-Renal complications: no further definition available.
-Diabetic retinopathy: ophthalmic complications; a chronic, pathological complication associated with diabetes mellitus, where retinal damages are incurred due to microaneurysms in the vasculature of the retina, progressively leading to abnormal blood vessel growth, and swelling and leaking of fluid from blood vessels, resulting in vision loss or blindness.
-Diabetic neuropathy: neurological complications; a chronic, pathological complication associated with diabetes mellitus, where nerve damages are incurred due to diabetic microvascular injury involving small blood vessels that supply these nerves, resulting in peripheral and/or autonomic nerve dysfunction.

Table S8 - Mendelian randomization results based on diabetes and diabetes-related complications as exposures and IL-6 as outcome

Exposure	Method	β (SE)	*P*	Numbr of SNPs (IV)	Pleiotropy test *
Type 2 diabetes	IVW	0.047 (0.071)	0.510	7	0.406
Type 2 diabetes with diabetic retinopathy	Wald	-0.059 (0.090)	0.509	1	NA
Type 1 diabetes	IVW	-0.025 (0.033)	0.445	7	0.897
Type 1 diabetes with diabetic angiopathy	Wald	0.009 (0.026)	0.727	1	NA
Type 1 diabetes with renal complications	IVW	-0.020 (0.021)	0.333	2	NA
Type 1 diabetes with diabetic retinopathy	IVW	-0.046 (0.029)	0.119	5	0.267
Type 1 diabetes with diabetic neuropathy	IVW	0.006 (0.011)	0.565	4	0.329

* Pleiotropy *P* value represents the *P* value of the intercept of Egger's regression.
IV, instrumental variable; IVW, inverse variance weighted random effects model; NA, not applicable; SE, standard error; SNP, single nucleotide polymorphism.

Definition of the diabetic complications in the FinnGen Biobank (https://risteys.finngen.fi/):
-Diabetic angiopathy: peripheral circulatory complications; vascular diseases that are associated with diabetes mellitus.
-Renal complications: no further definition available.
-Diabetic retinopathy: ophthalmic complications; a chronic, pathological complication associated with diabetes mellitus, where retinal damages are incurred due to microaneurysms in the vasculature of the retina, progressively leading to abnormal blood vessel growth, and swelling and leaking of fluid from blood vessels, resulting in vision loss or blindness.
-Diabetic neuropathy: neurological complications; a chronic, pathological complication associated with diabetes mellitus, where nerve damages are incurred due to diabetic microvascular injury involving small blood vessels that supply these nerves, resulting in peripheral and/or autonomic nerve dysfunction.

Fig. S1 – Study population

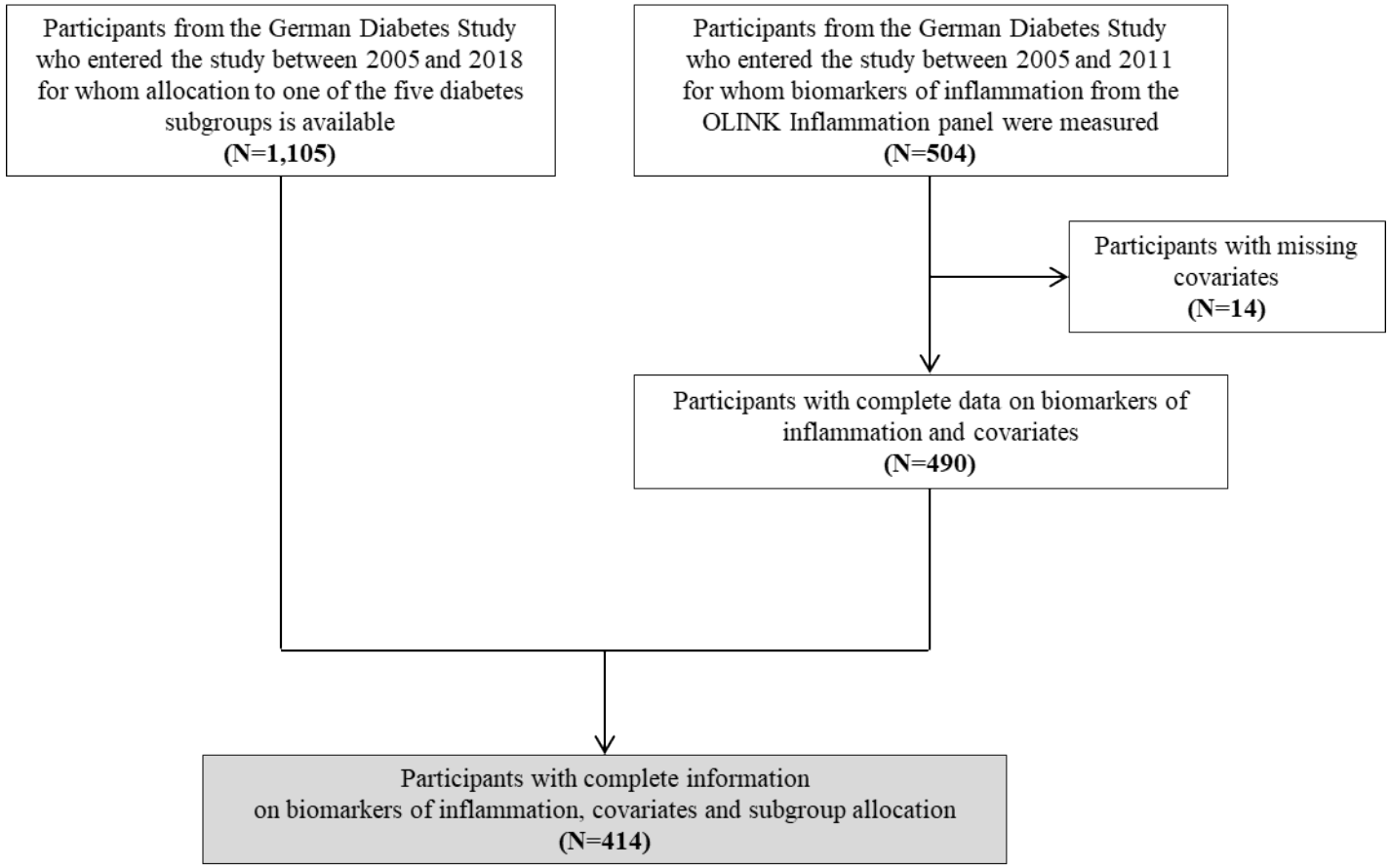
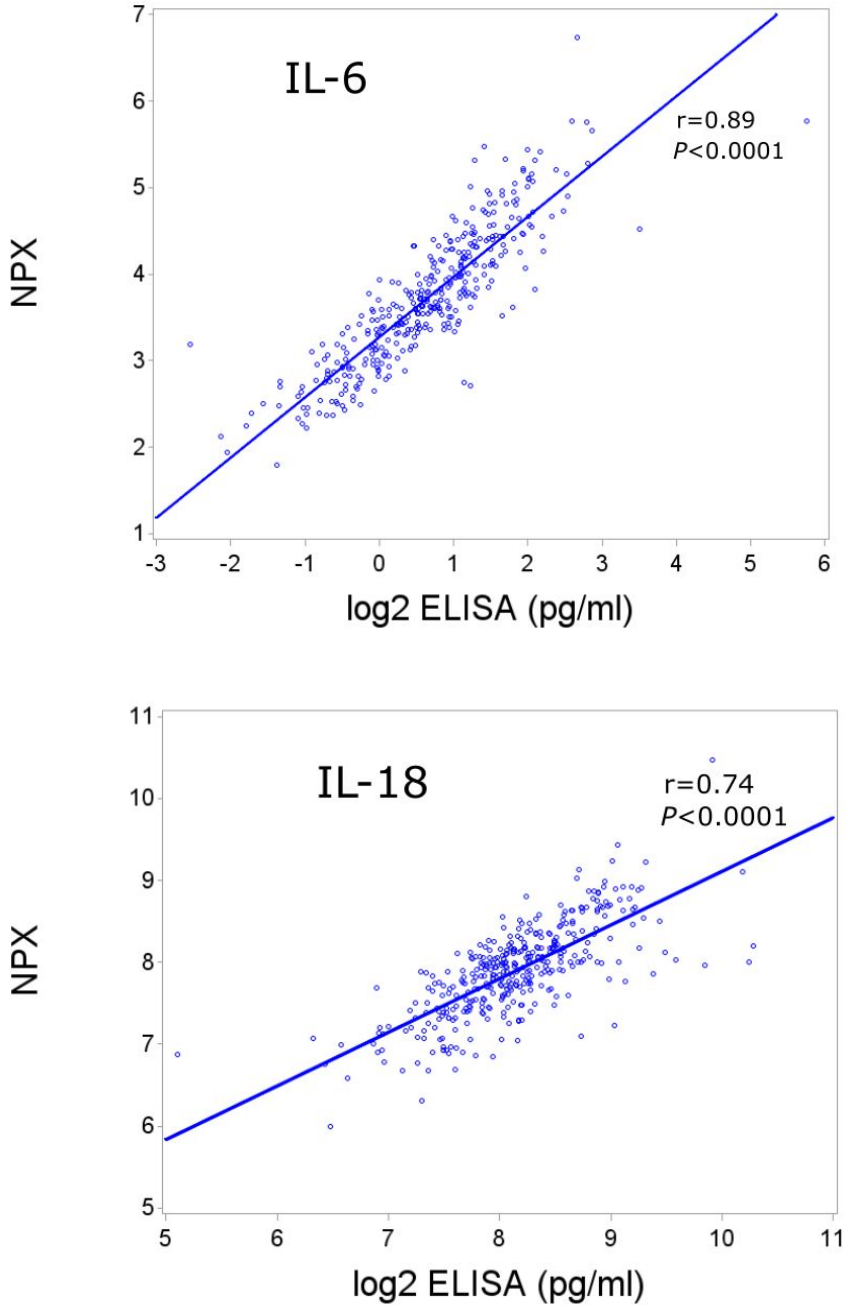


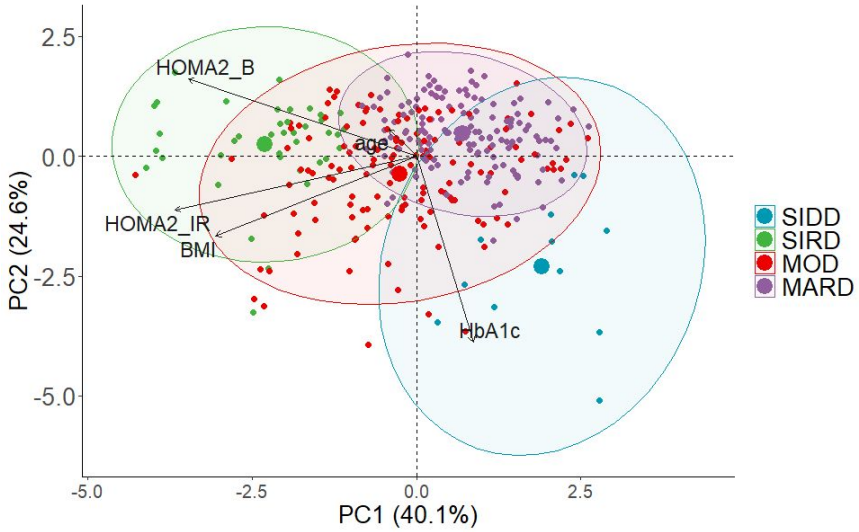
Fig. S2 – Correlation of IL-6 and IL-18 levels measured with ELISA and primer extension assay (PEA) technology in the German Diabetes Study



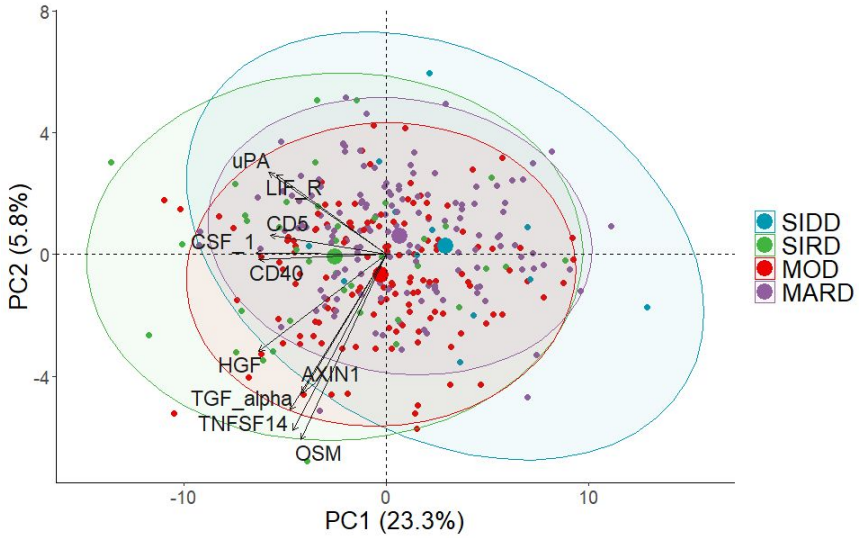
Correlations are estimated using Spearman correlation coefficients (r) and corresponding P values.

Fig. S3 - Principle component analysis based on clustering variables and biomarkers of inflammation

A



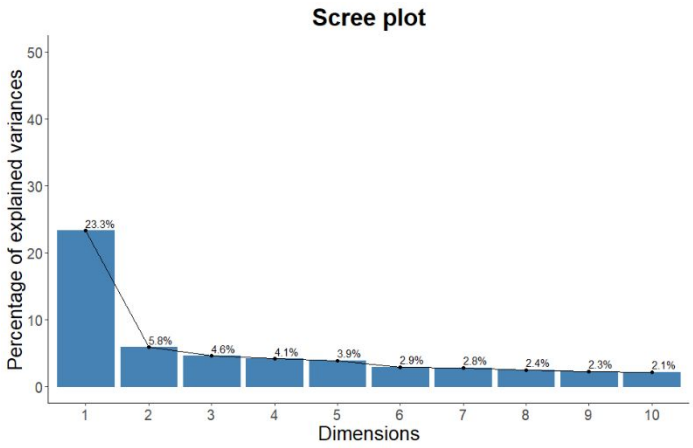
B



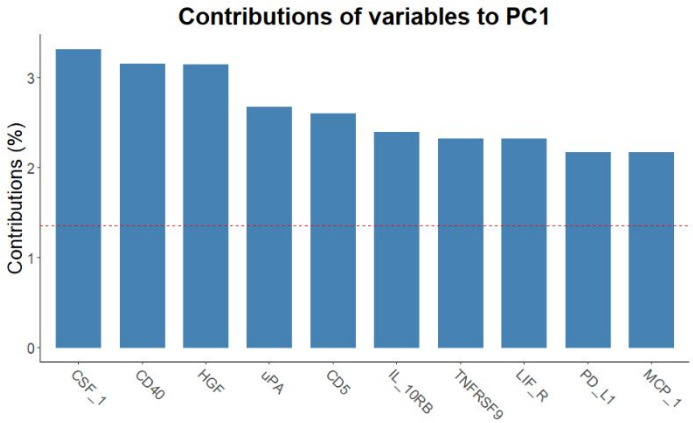
Principal component analysis (PCA) plot of the first two principal components (PC1 and PC2) based on clustering variables except GADA (A) or based on biomarkers of inflammation (B). Arrows indicate the variables with the most important contribution (highest values of cos2) to PC1 and PC2.

Fig. S4 - Contributions of biomarkers of inflammation to principal components

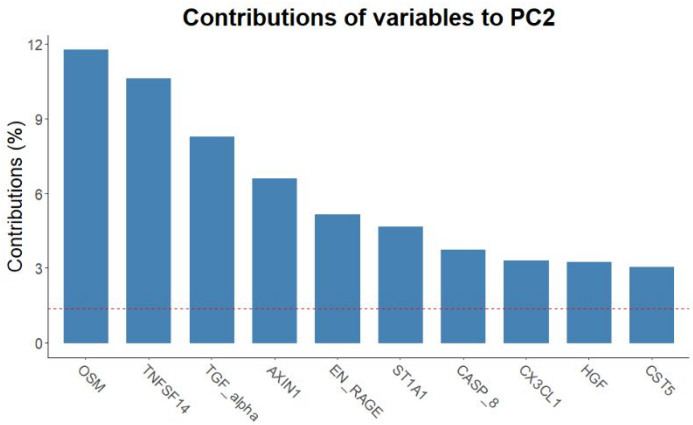
A



B



C



A. Scree plot of eigenvalues indicating the contributions of the first ten principal components to the explained variance of the PCA based on biomarkers of inflammation (see Fig. 3B). B, C. Plots showing the contributions of different biomarkers of inflammation to PC1 (B) and

PC2 (C). Red dashed lines indicate the expected value if contributions were uniform across biomarkers.