Modulating energy metabolism: pathophysiological aspects and novel interventions

Straat, M.E.

CHAPTER 3

Differences in inflammatory pathways between Dutch South Asians versus Dutch Europids with type 2 diabetes

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J Clin Endocrinol Metab 2023, in press.

ABSTRACT

Context

South Asians are more prone to develop type 2 diabetes (T2D) coinciding with earlier complications than Europids. While inflammation plays a central role in the development and progression of T2D, this factor is still underexplored in South Asians.

Objective

To study whether circulating mRNA transcripts of immune genes are different between South Asian versus Europid patients with T2D.

Design

Secondary analysis of two randomized controlled trials.

Participants

Dutch South Asian (n=45; age: 55±10 years, BMI: 29±4 kg/m^2) and Dutch Europid (n=44; age: 60±7 years, BMI: 32±4 kg/m^2) patients with T2D.

Main outcome measures

mRNA transcripts of 182 immune genes (microfluidic qPCR; Fluidigm Inc., USA) in fasted whole blood, ingenuity pathway analyses (Qiagen, USA).

Results

South Asians, compared to Europids, had higher mRNA levels of B cell markers [*CD19, CD79A, CD79B, CR2, CXCR5, IGHD, MS4A1, PAX5*; all FC>1.3, FDR<0.008] and IFN signaling genes [*CD274, GBP1, GBP2, GBP5, FCGR1A/B/CP, IFI16, IFIT3, IFITM1, IFITM3, TAP1*; all FC>1.2, FDR<0.05]. In South Asians, the IFN signaling pathway was the top canonical pathway (z-score 2.6, P<0.001) and this was accompanied by higher plasma IFN-γ levels (FC=1.5, FDR=0.01). Notably, the ethnic difference in gene expression was larger for females [20/182 (11%)] than males [2/182 (1%)].

Conclusions

South Asian patients with T2D show a more activated IFN signaling pathway compared to Europid patients with T2D, which is more pronounced in females than males. We speculate that a more activated IFN signaling pathway may contribute to the more rapid progression of T2D in South Asians compared with Europids.

INTRODUCTION

South Asians, originating from the Indian subcontinent and encompassing 20% of the world population, are at particularly high risk to develop type 2 diabetes (T2D). In high-income countries such as the Netherlands, South Asians have a two-to-four times higher risk of developing T2D compared to people from European origin, in the manuscript further called 'Europid' (1). Notably, at the moment of diagnosis, a high proportion of South Asian patients with T2D have a lower body mass index (BMI) and are at a younger age as compared to Europids. Additionally, in South Asians micro- and macrovascular complications of T2D evolve within a shorter duration after start of the disease (2, 3). Several factors have been proposed to underlie the increased susceptibility of South Asians to develop T2D compared to Europids, such as genetic predisposition, differences in lifestyle, central adiposity, low lean mass, low brown adipose tissue volume, and insulin resistance (4, 5). However, the high rate of T2D in South Asians cannot be fully explained by these factors alone.

Over the last decades, inflammation is increasingly acknowledged to play an important role in the pathogenesis of T2D, at least partly by accelerating insulin resistance (6-8). Accordingly, clinical studies have shown that anti-inflammatory treatments, such as the interleukin-1-receptor antagonist Anakinra and the anti-inflammatory compound salsalate, improve glycemic control in patients with T2D (9-12). Although anti-inflammatory therapy is thus regarded a promising strategy to improve T2D regulation, these studies have so far only been conducted in patients of Europid origin. Interestingly, previous studies support the presence of a more pro-inflammatory phenotype in South Asians compared with Europids. Concentrations of the nonspecific inflammatory marker C-reactive protein (CRP) are higher in healthy middle-aged South Asian compared with Europid men and women (13, 14). Furthermore, interleukin-6 (IL-6) levels are higher in healthy young South Asian men (15), and healthy middle-aged South Asian women than in matched Europids (16). These data thus support a possible pathophysiological role for inflammation in explaining the increased risk of the South Asian population to develop T2D. However, which aspects of the immune system may be differentially regulated in South Asians with T2D is currently unknown.

To further pinpoint such a role and to elucidate the possible clinical benefit of anti-inflammatory therapy to reduce T2D burden in the South Asian population, a detailed overview of the inflammatory state of South Asian patients with T2D is urgently needed. Therefore, the aim of this study was to investigate whether circulating mRNA transcripts of a broad range of immune related genes (using an untargeted approach by measuring

e.g. markers of T cells, B cells, NK cells and interleukins) are different between patients with T2D from Dutch South Asian versus Dutch Europid descent.

METHODS

Study design and participants

The current study is a secondary analysis of two previously performed double-blind, placebo controlled, randomized clinical trials that were both designed to investigate the effect of 26-weeks liraglutide treatment on cardiovascular endpoints in overweight and obese patients with T2D (17, 18). In the first trial (performed between 2013 and 2016), 49 Dutch Europid patients with T2D were included (17). In the second trial (performed between 2015 and 2018), 47 Dutch South Asian patients with T2D were included, of which South Asian ethnicity was based on being born and raised in the Netherlands and having four grandparents from South Asian descent (18). Due to missing samples, in the current study 44 Dutch Europid (19 females; 43%) and 45 Dutch South Asian (27 females; 60%) patients with T2D were included. For both trials, inclusion criteria were: body mass index (BMI) ≥ 23 kg/m^2; age 18–74 years old; and HbA1c ≥ 6.5% and ≤ 11.0% (≥ 47.5 and ≤ 96.4 mmol/mol). Patients were allowed to be treated with glucose-lowering medication (exclusively metformin, sulfonylurea derivatives and insulin), although with a stable dosage for at least 3 months prior to participation in the study. Patients were allowed to use antihypertensives and statins. Exclusion criteria were: use of other glucose-lowering medication than mentioned above; presence of renal disease; congestive heart failure according to New York Heart Association (NYHA) classification III–IV; uncontrolled hypertension (systolic blood pressure > 180 mmHg and/or diastolic blood pressure > 110 mmHg); or an acute coronary or cerebrovascular accident within 30 days prior to study inclusion. The trials were executed in the LUMC, the Netherlands, and were approved by the local ethics committee. Written informed consent was obtained from all subjects before inclusion. The trials were conducted in accordance with the principles of the revised Declaration of Helsinki and were registered at clinicaltrials.gov (NCT01761318 and NCT02660047, respectively).

Study procedures

This is a cross-sectional, single-visit study. The data used for the current secondary analyses were obtained at baseline before patients started their liraglutide treatment. During this visit, participants were fasted for at least 6 hours. Their medical history was interrogated to, amongst others, obtain information about their diabetes duration and medication use. Body weight and total fat mass were assessed using bioelectrical impedance analysis (Bodystat 1500, Bodystat Ltd., Douglas, UK). Visceral adipose tissue

(VAT) mass and abdominal subcutaneous adipose tissue (SAT) mass were assessed by MRI.

MRI protocol

The MRI protocol has been described in detail previously (19). In short, all participants underwent an MRI using a clinical 3 Tesla Ingenia whole-body MR system (Philips Medical Systems, Best, the Netherlands) at baseline and after 26 weeks of liraglutide treatment. Participants were scanned in supine position after at least 6 h fasting. Semi-automated segmentation of VAT and abdominal SAT was depicted by threshold-based inclusion of fat, with manual correction. VAT and SAT were calculated as mean area of fat in three slices.

Blood samples

Venous blood samples were drawn from the antecubital vein. To obtain plasma, blood samples were centrifuged, aliquoted, and stored at -80°C until batch-wise analyses. Plasma total cholesterol, HDL-cholesterol, triglyceride, and C-reactive protein (CRP) concentrations were measured on a Roche Modular analyzer (Roche Diagnostics, Mannheim, Germany). LDL-cholesterol was calculated according to the Friedewald formula. HbA1c was measured with ion-exchange high-performance liquid chromatography (HPLC; Tosoh G8, Sysmex Nederland B.V., Etten-Leur, the Netherlands). The commercially available protein biomarker panel "Target 96 Inflammation" from Olink proteomics (Olink Bioscience, Uppsala, Sweden) was used to measure interferon (IFN)-γ (20). Blood samples for RNA isolation were collected in PAXgene® Blood RNA tubes (BD Biosciences) and stored at -80°C following instructions from the manufacturer until batch-wise analyses.

RNA isolation and qPCR gene expression analyses

RNA isolation

Total RNA was extracted from whole blood samples in PAXgene® Blood RNA tubes using the automated PAXgene® Blood miRNA Kit (PreAnalitiX, Hombrechtikon, Switzerland) procedure, according to manufacturer's protocol. Briefly, cells were pelleted and lysed. Cell contents were treated with proteinase K and silica-based column extraction was performed, including on-column DNAse I treatment. Total RNA quantity was determined using Qubit RNA BR Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA).

cDNA synthesis and preamplification

cDNA was synthesized by performing reverse transcription of 50 ng RNA (incubation at 25°C for 5 minutes, 42°C for 30 minutes and 85°C for 5 minutes). Reverse Transcription Master Mix (Fluidigm, South San Francisco, CA, USA), containing M-MLV

reverse transcriptase, random hexamer, and oligo dT primers was used. cDNA was preamplified to increase the amount of input material needed for our high-throughput qPCR technique. For preamplification, we used a pool of the target TaqMan assays (Thermo Fisher Scientific, 0.2X each in TE buffer: 10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0) and Preamp Master Mix (Fluidigm) according to manufacturer's instructions. Thermal cycling conditions were: 95°C for 2 minutes followed by 14 cycles at 95°C for 15 seconds and 60°C for 4 minutes. Preamplified cDNA was diluted 1:5 in TE buffer and stored at -20°C prior to analysis.

High-throughput qPCR Gene Expression Analysis

mRNA transcripts of 182 genes were measured by high-throughput microfluidic qPCR using 96.96 IFC chips on the Biomark HD system (Fluidigm), as described by manufacturer. Each TaqMan Assay (20X, FAM-MGB; **Supplementary Table 1** (21)) was diluted in Assay Loading Reagent (Fluidigm) to a 10x assay mix. Sample mixes were prepared containing 1x TaqMan Universal PCR Master Mix (Thermo Fisher Scientific), 1x Sample Loading Reagent (Fluidigm) and 2.25 µL of preamplified cDNA. The 96.96 IFC chip was primed with Control Line Fluid (Fluidigm) and assay and sample mixes were loaded into the chip using the IFC Controller HX (Fluidigm). qPCR was performed with the Biomark HD using the following thermal cycling protocol: 95°C for 10 minutes, followed by 40 cycles at 95°C for 15 seconds and 60°C for 1 minute. Data was analyzed using Fluidigm Real-Time PCR Analysis Software (version 4.1.3). A cycle threshold (Ct) value ≤ 35 was determined as cutoff for reliable detection. Relative target gene expression was determined by calculating ΔCt using *GAPDH* as reference gene.

Ingenuity pathway analysis

Ingenuity pathway analysis (IPA; Qiagen, Redwood City, California, USA) was performed to assess transcriptional regulators and canonical pathways involved in observed differences in mRNA levels of immune genes between ethnicities. For each potential transcriptional regulator, the program calculates an overlap P value and activation z-score. The overlap P value is calculated using Fisher's Exact Test and indicates whether overlap between the genes in the dataset and genes regulated by the transcriptional regulator is significant. The activating z-score quantifies the predicted activation state of a transcriptional regulator. Overlap P values <0.01 and Z-scores greater than 2 are considered significant.

Statistical analyses

Statistical analyses were conducted using R (version 3.6.2, Team, 2019) and Prism 9 for Windows (version 9.0.1, 2021, GraphPad Software, LLC, San Diego, California, USA). Normal distribution of the baseline characteristics was tested using the Shapiro-Wilk

test. Dependent on whether the data followed normal Gaussian distribution, baseline characteristics between ethnicities and between sexes were compared using a two-tailed unpaired Student's t-test or the non-parametrical Mann-Whitney U test in R. mRNA levels of immune genes were expressed as ΔCT values on logarithmic scale with a base of 2 (log2). Detected proteins were generated as normalized protein expression (NPX) values on a log2 scale, with larger numbers representing higher protein levels in the sample. mRNA levels of immune genes and protein levels were compared between ethnicities and between sexes using an analysis of variance model (aov) in R with 'ethnicity' and/or 'sex' as between-subjects factor. For comparisons between ethnicities and/or sex, P values were corrected for multiple comparisons using Benjamini-Hochberg False Discovery Rate in R. A False Discovery Rate adjusted P value (FDR) of < 0.05 was considered statistically significant. Pearson correlation and simple linear regression were performed in Prism with mRNA levels as dependent outcome, and IFN-γ or fat mass percentage as independent outcomes. Here, P value of < 0.05 was considered statistically significant.

Since mRNA levels of immune genes were expressed as ΔCT values on a log2 scale, larger values represent lower mRNA levels in the sample. For the calculation of the log2 fold changes (log2FC) for volcano plots, the mean ΔCT value of Europid patients was subtracted from the mean ΔCT value of Dutch South Asian patients and multiplied by -1 to obtain the correct direction ([Dutch South Asian – Europid]*-1). Relative expression values (=fold changes) used in bar graphs were calculated using the ΔΔCT method, with the Europid patients as control group. Figures represent geometric mean and geometric standard deviation and all figures were prepared with Prism 9 for Windows (version 9.0.1, 2021, GraphPad Software, LLC, San Diego, California, USA). All supplemental figures are located in a digital research materials repository (21).

RESULTS

Baseline characteristics

Baseline characteristics are shown in **Table 1**, and in **Supplementary Table 2** (21) for males and females separately. As reported in a previous publication in which the primary end point of the current study was described (22), compared to Dutch Europids, Dutch South Asians had a lower body mass index (BMI, 29.4±4.0 *vs.* 32.3±4.0 kg/m^2) and lower LDL-cholesterol concentration (2.1±0.8 *vs.* 2.6±0.9 mmol/L). In addition, despite being younger (54.7±10.3 *vs.* 59.6±6.5 years), Dutch South Asians had a longer diabetes duration (17.4±9.9 *vs.* 10.7±6.4 years) and higher rates of retinopathy (51 *vs.* 9%) and macrovascular diseases (27 *vs.* 5%) than Dutch Europids (22).

Table 1. Baseline characteristics

	Dutch Europid (*n* = 44)	**Dutch South Asian (*n* = 45)**
Demographics		
Females, n, %	19, 43%	27, 60%
Age, years	59.6 ± 6.5	54.6 ± 10.3*
Diabetes duration, years	10.7 ± 6.4	17.4 ± 9.9**
Clinical parameters		
Weight, kg	97.0 ± 14.0	79.6 ± 11.9***
Length, cm	173.2 ± 8.9	165.6 ± 8.8***
BMI, kg/m^2	32.3 ± 4.0	29.4 ± 4.0**
Waist-height ratio	0.64 ± 0.06	0.61 ± 0.06*
Body fat percentage, %	37.1 ± 9.4	37.1 ± 9.2
VAT/SAT ratio	0.6 ± 0.3	0.6 ± 0.3
HbA1c, mmol/mol	66.1 ± 11.0	67.7 ± 11.5
HbA1c, %	8.2 ± 1.0	8.3 ± 1.1
Total cholesterol, mmol/L	4.8 ± 1.0	4.2 ± 1.0**
HDL-C, mmol/L	1.2 ± 0.3	1.2 ± 0.3
LDL-C, mmol/L	2.6 ± 0.9	2.1 ± 0.8**
CRP, mmol/L	3.1 ± 3.3	3.6 ± 4.1
Diabetic complications/comorbidity		
Diabetic retinopathy, n, %	4, 9%	23, 51%***
Diabetic nephropathy, n, %	11, 25%	10, 22%
Diabetic neuropathy, n, %	15, 34%	12, 27%
Macrovascular disease, n, %	2, 5%	12, 27%**
Concomitant medication use		
Metformin, mg/day	2047 ± 569	1750 ± 659*
Sulfonylurea, n, %	13, 30%	8, 18%
Insulin, n, %	28, 64%	34, 76%
Statin, n, %	36, 82%	34, 76%
Anti-hypertensive drug, n, %	34, 77%	32, 71%

Data are presented as mean ± standard deviation, unless specified otherwise. Specifically, in the section of diabetic complications/comorbidities, data are presented as the number of patients who reported to have these complications and the percentage of all. P values indicate differences between Dutch Europid versus Dutch South Asian patients. *P<0.05, **P<0.01, ***P<0.001.
BMI, body mass index; CRP, C-reactive protein, HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.

Circulating mRNA levels of B cell markers and IFN signaling genes are higher in Dutch South Asians compared with Dutch Europids with T2D

mRNA levels of 30/182 (16%) immune related genes were significantly different (FDR < 0.05) between Dutch South Asians and Dutch Europids (**Figure 1**). Among those, mRNA levels of 3 genes were significantly lower in Dutch South Asians (scavenger receptor *MARCO,* anti-inflammatory cytokine *IL10,* and inflammasome component *NLRP2*; all FC<0.7, FDR<0.04), whereas mRNA levels of 27 genes were higher in Dutch South Asians compared to Dutch Europids. Specifically, mRNA levels of the apoptosis involved *CASP8*, oncogene *AKT1*, T cell subset marker *CD3E*, natural killer cell marker *KLRC2/3*, cytotoxicity marker *GZMA*, and pattern recognition receptors *TLR7*, *TLR10*, *NOD1*, and *NOD2* were higher in Dutch South Asians than in Dutch Europids (all FC>1.2, FDR<0.05).

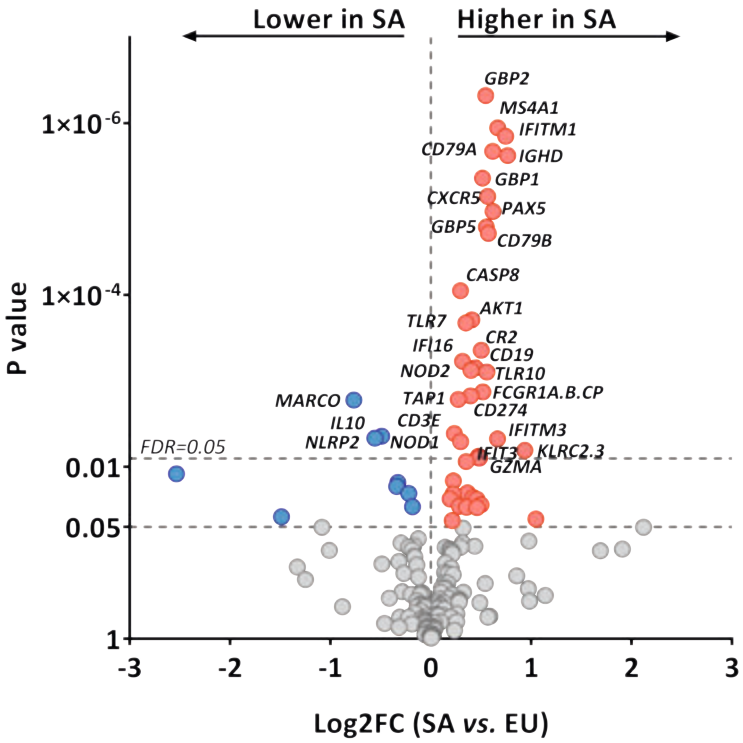

Figure 1. Differences in circulating mRNA levels of immune genes between Dutch South Asian vs. Dutch Europid patients with type 2 diabetes.

Volcano plot showing the differences in circulating mRNA levels of immune related genes between Dutch South Asian and Dutch Europid patients with type 2 diabetes. The X-axis shows the log2FC between Dutch South Asian and Dutch Europid patients, the Y-axis shows the P value. P values were obtained from one-way ANOVA and thereafter corrected using Benjamini–Hochberg's false discovery rate procedure. The top dashed line represents FDR=0.05.

EU, Dutch Europid patients; FDR, false discovery rate adjusted p value; Log2FC, log2 fold change; SA, Dutch South Asian patients.

Additionally, we found a clear pattern in which mRNA levels of 8/10 (80%) measured B cell markers (*CD19*, *CD79A*, *CD79B*, *CR2*, *CXCR5*, *IGHD*, *MS4A1*, *PAX5*; all FC>1.4, FDR<0.008; **Figure 2A**) and 10/25 (40%) measured IFN signaling genes (*CD274, FCGR1A/B/CP, GBP1, GBP2, GBP5, IFI16, IFITM1, IFITM3, IFIT3, TAP1*; all FC>1.2, FDR<0.05; **Figure 2B**) were higher in Dutch South Asians than in Dutch Europids.

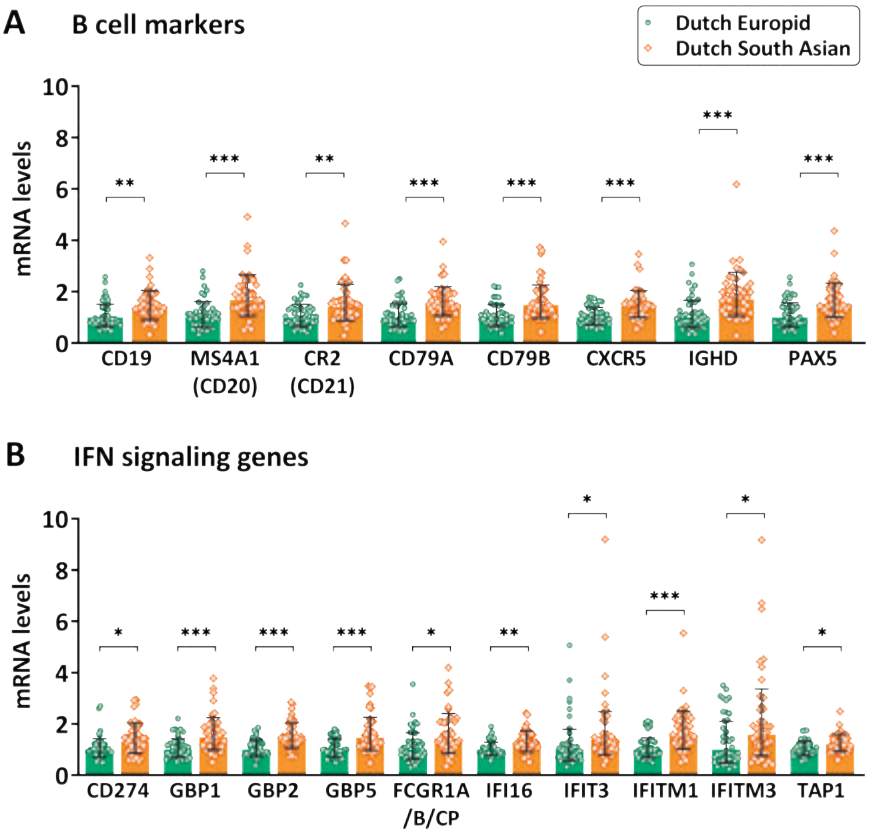

Figure 2. Differences in mRNA levels of B cell markers and IFN signaling genes in Dutch South Asian compared to Dutch Europid patients with type 2 diabetes.
Bar charts showing mRNA levels of B cell markers (A) and IFN signaling genes (B), that are different between Dutch South Asian vs. Dutch Europid type 2 diabetes patients (FDR<0.05). mRNA levels are represented relative to levels of Europids, with geometric mean and geometric standard deviation. *FDR<0.05, **FDR<0.01, ***FDR<0.001 (one-way ANOVA, thereafter corrected using Benjamini-Hochberg's false discovery rate procedure).

The IFN signaling pathway is the top canonical pathway that could explain the observed ethnic difference in mRNA levels of immune related genes

IPA was performed to identify transcriptional regulators and canonical pathways that could explain the observed ethnic differences in mRNA levels of immune genes. In total, 469 potential transcriptional regulators were identified with a statistically significant overlap (P<0.01) between the genes in our dataset and genes known to be regulated by these transcriptional regulators. Among those, 60 transcriptional regulators had a z-score of > 2 or <-2. The top 20 hits with the highest z-score are shown in **Figure 3A**. IFN-γ had the highest z-score (z-score: 4.1, P<0.001), followed by IFN-α (z-score: 3.7, P<0.001), IFN regulatory factor 7 (IRF7; z-score: 3.4, P<0.001), IRF1 (z-score: 3.4, P<0.001), IRF3 (z-score: 3.3, P<0.001), and IFN-α/β receptor (IFNAR; z-score: 3.2, P<0.001). In addition, using ingenuity pathway analysis, 68 significant canonical pathways (P<0.01) were identified, of which only 4 pathways had a z-score of > 2 (**Figure 3B**). These top canonical pathways were: IFN signaling (z-score: 2.6, P<0.001), role of pattern recognition receptors in recognition of bacteria and viruses (z-score: 2.6, P<0.001), Th1 pathway (z-score: 2.4, P<0.001), and systemic lupus erythematosus in B cell signaling pathway (z-score: 2.3, P<0.001). Thus, both in the transcriptional regulators and canonical pathways, IFN signaling pathways appeared as a top hit.

Moreover, plasma protein levels of IFN-γ were higher in Dutch South Asians compared to Dutch Europids (FC=1.5, FDR=0.01; **Supplementary figure 1A**) (21). IFN-γ protein levels were positively associated with mRNA levels of several IFN signaling genes that were different between Dutch South Asians and Dutch Europids (**Supplementary figure 1B)** (21). In both ethnicities, IFN-γ protein levels were positively associated with mRNA levels of *CD274*, *GBP1*, *GBP5*, and *IFIT3*. Additionally, in Dutch South Asians only, IFN-γ protein levels were positively associated with mRNA levels of *GBP2* and *FCGR1A/B/CP*, and in Dutch Europids only, with mRNA levels of *IFITM3* (**Supplementary figure 1B**) (21).

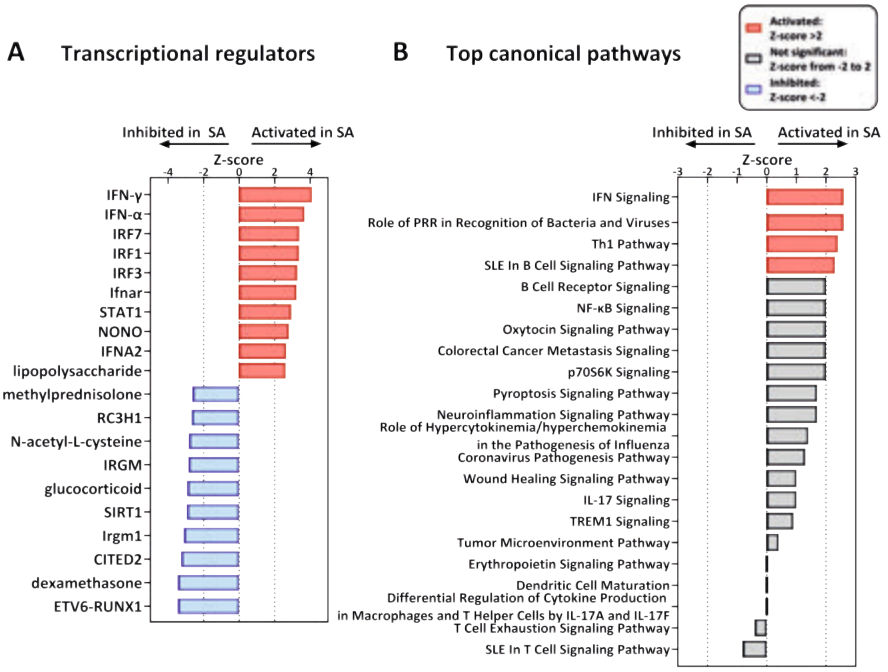

Figure 3. Transcriptional regulators and top canonical pathways that could explain the observed ethnic difference in mRNA levels of immune genes.
(A) The top 10 upregulating, and top 10 downregulating transcriptional regulators with a statistically significant overlap (P<0.01) and a z-score of > 2 or <-2. The y-axis shows the name of the transcriptional regulators, the x-axis shows the z-score.
(B) Top canonical pathways with a statistically significant overlap (P<0.01) and a z-score of ≥2. Those with a z-score of > 2 in red. The y-axis shows the name of the pathway, the x-axis shows the z-score. Transcriptional regulators and top canonical pathways are obtained from ingenuity pathway analyses, performed on the list of genes with a differential expression (P<0.05) between Dutch South Asians and Dutch Europids, with as input the log2FC differences in mRNA levels.

The ethnic differences in immune mRNA levels are larger in females than in males

Interestingly, although we did not observe an interaction between sex and ethnic difference in mRNA levels (P>0.05), the ethnic difference in mRNA levels of immune genes was more pronounced in females (20/182, 11%) than males (2/182 genes, 1%; **Figure 4A** and **B**). In Dutch South Asian males, mRNA level of *ETV7* was lower than in Europid males (FC=0.4, FDR=0.05), and B cell marker *IGHD* was higher (FC=1.7, FDR=0.05; **Figure 4A**). In Dutch South Asian females, mRNA level of *MARCO* was lower than in Europid females (FC=0.4, FDR=0.01), whereas levels of *CASP8*, *NOD2*, *ZNF532*, *LAG3* and *AKT1* were higher (all FC>1.3, FDR<0.05; **Figure 4B**). Moreover, 7/10 B cell markers (*CD19*,

CD79A, *CD79B*, *CXCR5*, *IGHD*, *MS4A1*, *PAX5*; all FC>1.5, FDR<0.04) and 7/25 IFN signaling genes (*GBP1, GBP2, GBP5, IFI16, IFITM1, IFITM3, TAP1*; all FC>1.5, FDR<0.05) were higher in Dutch South Asian females (**Figure 4B**).

Next, we performed correlation analyses between baseline characteristics and mRNA levels of B cell markers, IFN signaling genes, and IFN-γ protein level, for males and females separately (**Table 2**). Only a few genes showed an association with baseline characteristics (P<0.05), however, none of the correlations remained significant after False Discovery Rate correction for multiple comparisons.

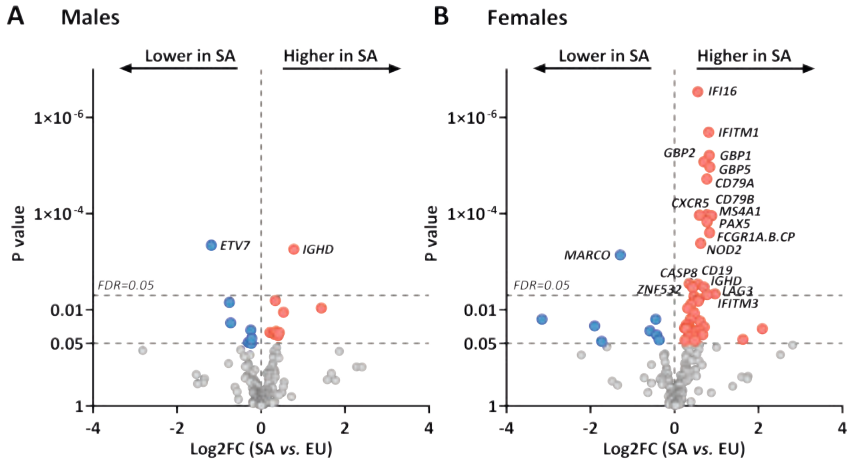

Figure 4. Differences in mRNA levels of immune genes between Dutch South Asian vs. Dutch Europid males and females with type 2 diabetes.
(A+B) Volcano plot showing the differences in mRNA levels of immune related genes between Dutch South Asian and Dutch Europid type 2 diabetes patients, in males (A) and females (B). The X-axes show the log2FC between Dutch South Asian and Dutch Europid patients, the Y-axes show the P value. P values were obtained from one-way ANOVA and thereafter corrected using Benjamini–Hochberg's false discovery rate procedure. The top dashed line represents FDR=0.05.
EU, Dutch Europid patients; FDR, false discovery rate adjusted p value; Log2FC, log2 fold change; SA, Dutch South Asian patients.

Table 2. Associations between baseline characteristics and mRNA levels of B cell markers, IFN signaling genes, and IFN-γ protein levels.

	Males														Females													
	Dutch Europid							Dutch South Asian							Dutch Europid							Dutch South Asian						
	Age	DD	BMI	Body fat %	Waist-height ratio	HbA1c	Metf. dose	Age	DD	BMI	Body fat %	Waist-height ratio	HbA1c	Metf. dose	Age	DD	BMI	Body fat %	Waist-height ratio	HbA1c	Metf. dose	Age	DD	BMI	Body fat %	Waist-height ratio	HbA1c	Metf. dose
B cell markers																												
CD19	-0.14	0.39	-0.03	-0.21	0.07	0.18	0.13	-0.12	0.17	0.12	-0.04	0.10	-0.14	0.17	-0.10	0.27	0.13	0.10	0.19	-0.41	0.11	-0.08	-0.01	0.16	0.11	0.20	-0.25	**0.47***
MS4A1(CD20)	-0.12	0.23	0.20	-0.12	0.21	0.03	-0.09	-0.22	-0.03	0.25	0.18	0.14	0.00	0.06	0.13	-0.27	0.30	0.37	0.43	-0.31	0.05	-0.09	-0.05	0.04	-0.06	0.10	-0.26	0.34
CR2(CD21)	-0.35	0.17	0.01	-0.17	-0.02	0.26	-0.05	-0.13	-0.25	0.19	0.19	0.19	0.05	-0.16	**-0.58****	0.17	**0.59****	0.22	0.38	0.13	-0.15	-0.26	-0.15	0.15	0.01	0.16	-0.19	0.33
CD79A	-0.24	0.24	0.07	-0.21	0.11	0.17	-0.02	-0.12	0.02	0.16	0.12	-0.22	-0.17	0.49	0.12	0.24	0.33	0.28	0.30	**-0.52***	0.15	-0.11	-0.06	0.11	0.04	0.16	-0.21	0.43
CD79B	-0.14	0.28	-0.01	-0.20	0.06	0.11	0.00	-0.07	0.26	-0.23	-0.21	-0.07	-0.09	**0.25***	-0.02	0.24	0.19	0.03	0.42	-0.44	0.01	-0.09	0.00	0.17	0.11	0.21	-0.11	**0.41***
CXCR5	-0.19	0.33	0.01	-0.15	0.06	0.08	0.04	-0.07	0.15	-0.01	-0.19	0.22	-0.23	-0.09	-0.15	0.17	0.33	0.25	0.24	-0.35	0.05	-0.24	-0.09	0.27	0.15	0.09	-0.21	**0.37***
IGHD	-0.39	0.24	0.15	-0.18	0.09	0.04	-0.05	0.11	0.18	0.12	-0.06	0.21	-0.01	0.09	-0.05	-0.02	0.34	0.25	**0.50***	-0.41	0.01	-0.19	-0.09	0.28	0.17	0.18	-0.21	0.37
PAX5	-0.23	0.24	0.12	-0.15	0.12	0.14	-0.03	0.04	0.25	0.29	0.24	0.36	0	0.19	-0.09	-0.12	0.4	0.37	0.33	-0.32	0.07	-0.1	0.01	0.07	-0.03	0.14	-0.24	0.34
IFN signaling genes + IFN-γ																												
CD274	0.21	0.25	0.07	0.08	0.20	0.12	0.28	-0.06	0.25	0.30	0.09	0.27	0.23	-0.40	0.10	-0.08	-0.23	-0.18	-0.18	0.02	0.07	-0.10	0.08	**0.49****	**0.39***	0.32	-0.20	0.15
GBP1	0.13	0.10	0.09	-0.05	0.03	0.02	0.24	-0.13	-0.07	-0.02	0.02	0.01	0.03	-0.16	0.21	-0.08	-0.07	-0.14	0.15	0.11	0.37	0.01	0.26	0.24	0.03	0.33	-0.23	0.25
GBP2	-0.14	-0.16	0.34	0.15	0.30	-0.10	0.03	-0.21	-0.45	0.22	0.14	0.14	-0.05	-0.38	0.13	-0.06	0.03	-0.03	0.26	-0.10	0.01	0.04	0.11	0.25	0.13	0.33	-0.27	0.31
GBP5	0.20	0.20	0.11	-0.15	0.06	0.09	0.14	-0.14	-0.13	0.19	0.19	0.17	-0.16	-0.18	0.25	-0.08	-0.18	-0.16	-0.03	0.06	0.19	0.11	0.29	0.30	0.24	**0.48***	-0.27	0.19
FCGR1A/B/CP	0.03	-0.09	-0.11	-0.04	0.08	-0.13	0.17	-0.04	0.06	0.24	0.20	0.24	0.07	-0.13	-0.22	-0.02	0.18	0.12	0.30	0.00	0.33	-0.25	-0.20	**0.49****	**0.48***	0.35	-0.07	0.15
IFI16	-0.06	0.13	-0.01	-0.05	0.15	-0.24	0.28	-0.45	-0.29	0.31	0.06	0.13	-0.17	-0.28	0.08	0.05	0.05	-0.29	0.24	0.11	0.29	-0.31	-0.17	0.23	0.13	0.16	-0.19	0.16
IFIT3	0.14	0.04	0.03	0.00	0.05	-0.30	0.15	0.26	0.11	0.28	0.44	0.42	0.04	-0.18	0.29	-0.03	0.16	-0.12	0.31	-0.06	0.16	-0.35	-0.15	0.27	0.00	0.19	-0.21	0.09
IFITM1	0.01	-0.01	-0.25	-0.24	-0.09	-0.22	-0.05	-0.30	-0.12	-0.07	0.10	-0.12	0.18	0.01	-0.19	-0.03	-0.01	-0.22	0.08	-0.09	0.26	-0.20	-0.07	0.16	0.12	-0.02	-0.32	0.37
IFITM3	0.06	-0.01	-0.39	-0.25	-0.24	-0.34	0.11	0.11	0.05	**0.53***	**0.53***	**0.58***	0.26	-0.15	0.13	-0.23	0.35	0.23	**0.54***	-0.28	0.16	-0.21	-0.12	-0.09	-0.20	-0.31	-0.13	0.21
TAP1	-0.06	-0.01	0.23	0.17	0.25	-0.26	0.11	-0.18	**-0.47***	0.15	0.09	-0.04	-0.45	-0.19	-0.04	**-0.53***	0.21	0.27	0.18	-0.11	-0.11	0.01	0.04	0.10	-0.01	0.18	-0.25	0.16
IFN-γ (protein)	**0.52****	0.02	-0.24	-0.20	-0.27	0.00	0.18	0.01	0.16	0.20	0.29	0.26	0.21	0.03	0.08	-0.13	-0.10	-0.08	-0.15	-0.07	0.20	-0.08	0.07	0.32	0.29	0.38	-0.38	0.15

Values indicate Pearson r. Correlations that were significant before correction using the Benjamini–Hochberg's false discovery rate procedure are highlighted in bold (P<0.05). None of the correlations remained significant after correction. *P<0.05, **P<0.01. BMI, body mass index; DD, diabetes duration; HbA1c, hemoglobin A1c; Metf., Metformin.

Values indicate Pearson r. Correlations that were significant before correction using the Benjamini–Hochberg's false discovery rate procedure are highlighted in bold (P<0.05). None of the correlations remained significant after correction. *P<0.05, **P<0.01. BMI, body mass index; DD, diabetes duration; HbA1c, hemoglobin A1c; Metf., Metformin.

DISCUSSION

In this study, we compared mRNA levels of a large panel of immune related genes between Dutch South Asian and Dutch Europid patients with T2D. We found that mRNA levels of 8/10 (80%) measured B cell markers and 10/25 (40%) measured IFN signaling genes in addition to IFN-γ protein levels were higher in Dutch South Asians compared to Dutch Europids. IPA showed that the IFN signaling pathway was the most activated canonical pathway and IFN-γ the top activating transcriptional regulator explaining these differences. Accordingly, IFN-γ protein levels were higher in Dutch South Asians compared to Dutch Europids. These ethnic differences were more pronounced in females than in males. We hypothesize that an enhanced IFN signaling pathway may contribute to the more severe disease progression and accelerated risk for T2D associated complications that is generally found in the South Asian compared to the Europid population.

The finding that the IFN signaling pathway is more activated in Dutch South Asian, compared to Dutch Europid patients with T2D is in seeming contrast with one of our previous findings. In overweight pre-diabetic men, we found lower mRNA levels of several type I IFN signaling genes in subcutaneous white adipose tissue and skeletal muscle biopsies of South Asian compared with Europid men, without differences in blood mRNA levels of IFN signaling genes (23). Although both type I IFNs and IFN-γ (*i.e.*, type II IFN) regulate the antiviral response, they are in fact structurally unrelated, bind to a different receptor, and have distinct physiological functions (24, 25). In turn, both studies point towards dysregulated IFN signaling in metabolically comprised Dutch South Asians, perhaps starting with impaired type I IFN signaling in metabolic tissues in subjects with prediabetes, followed by an overall more activated inflammatory response including accelerated IFN signaling pathways once the disease progresses.

Indeed, the involvement of IFN signaling in the development and progression of T2D has been supported by previous preclinical and clinical studies. In pre-diabetic obese mice, virally induced IFN-γ (using murine cytomegalovirus) drives the progression from pre-diabetes to T2D, by causing insulin resistance in skeletal muscles through downregulation of the insulin receptor (26). Also in primary human adipocytes, IFN-γ induces insulin resistance by downregulating the insulin receptor, as well as insulin receptor substrate-1 and GLUT4 (27). These pre-clinical findings are corroborated by an observational study showing that patients with T2D, compared to controls, have higher IFN-γ levels that positively correlate with HbA1c levels (28). Moreover, in a cohort study consisting of 157 overweight Dutch Europids, IFN-stimulated genes were found to be upregulated in the whole blood transcriptome of insulin-resistant compared with

insulin-sensitive individuals (29). These data thus suggest that IFN signaling pathways may play a role in the pathophysiology of T2D. Unfortunately, in the current study we did not study the severity of peripheral insulin resistance (*e.g.* via hyperinsulinemic euglycemic clamp). This would have enabled us to study possible associations of IFN signaling genes with insulin resistance. Notably, in patients with T2D, IFN-γ is also shown to be positively related to diabetes-associated complications such as nephropathy (30) and diabetic foot ulcers (31). Thus, a higher activation of the IFN signaling pathway could contribute to the increased risk of South Asians to develop T2D, but also a more severe T2D progression with a higher risk of T2D associated complications. Nonetheless, we cannot exclude that a higher activation of the IFN signaling pathway is reflective of a longer disease progression in South Asians with T2D, since the Dutch South Asians included in our study had a longer T2D duration compared to the Dutch Europids.

Next to higher expression of IFN signaling genes, we found higher mRNA levels of B cell markers, and lower mRNA levels of IL-10, in Dutch South Asian compared to Europid patients with T2D. B cells can contribute to insulin resistance via antigen presentation to T cells, changes in cytokine secretion, and pathogenic antibody production, thereby activating T cells (that secrete *e.g.* IFN-γ) and polarizing macrophages towards a pro-inflammatory phenotype (32-34). B cell deficient mice on a high-fat diet, compared to wild-type controls on the same diet, display lower blood glucose levels and less insulin resistance (33), accompanied with decreased systemic- and adipose tissue inflammation, and decreased adipose tissue IFN-γ expression (34). In healthy individuals, B cells are an important source of anti-inflammatory IL-10 secretion, and circulating IL-10 is positively associated with insulin sensitivity (35). On the other hand, an impaired IL-10 response upon a pro-inflammatory stimulus is related to the presence of metabolic syndrome and T2D in old individuals (36), and B cells from patients with T2D show diminished IL-10 secretion upon stimulation of its toll-like receptors (37). In summary, B cells can become involved in inflammation and insulin resistance, amongst others, by decreasing IL-10 secretion, and activating T cells that produce IFN-γ (32). The results of the current study suggest that this inflammatory pathway is enhanced in Dutch South Asian compared to Europid patients with T2D and future studies should investigate whether the phenotype of B cells in South Asians indeed associates with enhanced insulin resistance.

We observed that the ethnic differences in immune mRNA levels were more pronounced in female patients with T2D than in male patients with T2D. Generally, premenopausal females are at lower risk to develop cardiometabolic diseases compared to men of the same age. However, after the menopause or once T2D has developed, this sex-dependent benefit diminishes and the cardiometabolic risk of females accelerates (38, 39). Consequently, postmenopausal females with T2D are at greater risk to develop

cardiovascular comorbidities, compared to age-matched males with T2D (39, 40). Interestingly, the age at menopause differs across populations, with lower menopausal ages reported among South Asians (*i.e.*, ranging from 44 to 49 years old), than Europids (*i.e.*, 50 to 54 years old) (41, 42). In this study, we cannot exclude that more South Asian females have reached the postmenopausal state than Europid females, which, together with their earlier diabetes onset, could result in a more disturbed immune system. Another factor to consider when speculating about the origin of the enhanced circulating mRNA levels of IFN signaling genes in Dutch South Asian females is adipose tissue. Total body fat percentage was slightly lower in Dutch South Asian females than in Dutch Europid females, and did overall not correlate with mRNA levels of IFN signaling genes, indicating that total fat mass might not explain the found differences. However, previously, it has been reported that healthy young South Asians, compared to Europids, have larger subcutaneous adipocytes (43, 44) and increased adipose tissue macrophage infiltration (45), regardless of total body fat or visceral fat content. Hypertrophic adipocytes that are overloaded with triglycerides are at risk for hypoxia (46). Induction of hypoxia-inducible factor-1 (HIF-1) and endoplasmic reticulum stress causes adipocyte cell death as well as the infiltration of immune cells from the innate and adaptive immune system, such as B cells, T cells, and macrophages resulting in a local and systemic pro-inflammatory environment (47, 48). Hypothetically, dysfunctional adipose tissue, regardless of adipose tissue mass, may be driving insulin resistance and the progression towards T2D.

One of the strengths of our study is the large array of immune related genes that were measured in both males and females, allowing to detect transcriptional regulators and canonical pathways driving the observed differences in mRNA levels. Unfortunately, mRNA levels of immune genes were only measured in whole blood samples, without isolating cells using flow cytometry analyses, lacking the ability to relate mRNA expression data with immune cell numbers and composition in blood. Furthermore, this could have been followed by *ex vivo* stimulation experiments on blood cells derived from both ethnicities to study functionality of immune cells. Moreover, we did not measure inflammatory molecules in insulin target tissues, such as white adipose tissue and skeletal muscle, as a result of which we cannot relate differences in circulating immune genes with those in metabolically active tissues. Similarly, we lack information on the menopausal state of females included in the current study. Concerning the study design, it remains a challenge to optimally match Dutch South Asian and Europid individuals. In this study, Dutch South Asians were younger, had a lower BMI, but had a longer diabetes duration, meaning a longer diabetic treatment period, which may have influenced the results. All these mentioned baseline differences can thus be confounders in the current study and results should be interpreted with this in mind.

In addition, although medication use did not differ between ethnicities, we cannot exclude that use of prior medication (metformin, sulfonylurea, insulin, antihypertensives and statins) may have influenced the results. Lastly, it is important to consider that an ethnicity is defined by cultural traditions. Therefore, we cannot exclude the influence of differences in behavior and lifestyle on the results in this study.

In conclusion, we show that circulating mRNA levels of IFN signaling genes and B cell markers are higher in Dutch South Asian than in Europid patients with T2D. We propose that increased inflammation, involving both B cells and IFN signaling pathways, in Dutch South Asian patients with T2D, is possibly contributing to the rapid progression of T2D and its complications in this population. Only future intervention studies can show whether targeting the IFN pathway for the treatment of T2D using anti-inflammatory therapies will be beneficial in the Dutch South Asian population. In this respect, the relatively novel antidiabetics glucagon-like peptide-1 receptor agonists and sodium-glucose cotransporter-2 inhibitors, that have been shown to exert cardiorenal protective effects, are particularly interesting.

FUNDING

Novo Nordisk A/S (Bagsvaerd, Denmark), the Dutch Diabetes Foundation (2015.81.1808 to M.R.B.), a Maria Zambrano fellowship by the Ministerio de Universidades y la Unión Europea – NextGenerationEU (RR_C_2021_04 to B.M.T.), the Netherlands Cardiovascular Research Initiative: an initiative with support of the Dutch Heart Foundation (CVON2017 GENIUS-2 to P.C.N.R.).

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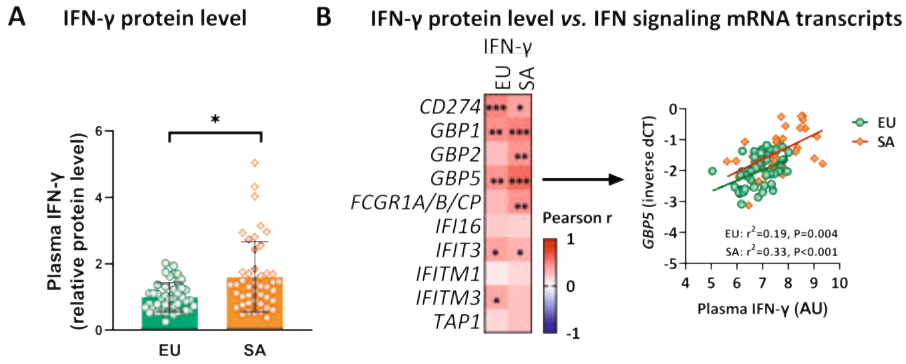
SUPPLEMENTAL DATA



Supplementary figure 1. IFN-γ protein level in Dutch South Asian vs. Dutch Europid patients with type 2 diabetes, and its association with mRNA levels of IFN signaling genes.
(A) IFN-γ protein level in Dutch South Asian (SA) relative to Dutch Europid (EU) patients with type 2 diabetes. *FDR<0.05 (one-way ANOVA, corrected using Benjamini-Hochberg's false discovery rate procedure). (B) Pearson correlations between mRNA levels of IFN signaling genes (inverse dCT) and IFN-γ protein levels (arbitrary units; AU). Color represents Pearson r, with positive correlations denoted by red color. *P<0.05, **P<0.01, ***P<0.001. Since the correlation plots between IFN-γ protein level and mRNA levels of CD274, GBP1, GBP5 and IFIT3 all looked similar, as example, the correlation plots between IFN-γ and mRNA levels of GBP5 are shown.

Supplementary table 1. Overview of all immune-related genes (n=182) measured using TaqMan.

Target genes	
Gene	**TaqMan Assay**
AIRE	Hs00230829_m1
AKT1	Hs00178289_m1
AKT3	Hs00987350_m1
AREG	Hs00950669_m1
ASAP1	Hs00987469_m1
BATF2	Hs00912737_m1
BCL2	Hs00608023_m1
BMP6	Hs01099594_m1
BPI	Hs01552756_m1
C1QA	Hs00381122_m1
C1QB	Hs00608019_m1
C1QC	Hs00757779_m1
CAMTA1	Hs01051596_m1
CASP8	Hs01018151_m1
CCL11	Hs00237013_m1
CCL13	Hs00234646_m1
CCL19	Hs00171149_m1
CCL2	Hs00234140_m1
CCL22	Hs01574247_m1
CCL3	Hs00234142_m1
CCL4	Hs99999148_m1
CCL5	Hs00982282_m1
CCR7	Hs01013469_m1
CD14	Hs00169122_g1
CD163	Hs00174705_m1
CD19	Hs99999192_m1
CD209	Hs01588349_m1
CD27	Hs00386811_m1
CD274	Hs00204257_m1
CD28	Hs01007422_m1
CD38	Hs01120071_m1
CD3E	Hs01062241_m1
CD4	Hs01058407_m1
CD79A	Hs00998119_m1
CD79B	Hs00236881_m1
CD8A	Hs00233520_m1

Target genes	
Gene	**TaqMan Assay**
CLEC7A	Hs00224028_m1
CR2	Hs00153398_m1
CTLA4	Hs00175480_m1
CX3CL1	Hs00171086_m1
CXCL10	Hs00171042_m1
CXCL13	Hs00757930_m1
CXCL9	Hs00171065_m1
CXCR5	Hs00173527_m1
DSE	Hs00203441_m1
EGF	Hs01099990_m1
ETV7	Hs00903229_m1
FASLG	Hs00899442_m1
FCGR1A/B/CP	Hs00174081_m1
FCGR3A	Hs04188274_m1
FLCN	Hs00376065_m1
FOXP3	Hs01085834_m1
FPR1	Hs00181830_m1
GATA3	Hs00231122_m1
GBP1	Hs00977005_m1
GBP2	Hs00894837_m1
GBP5	Hs00369472_m1
GCK	Hs01564555_m1
GLP1R	Hs00157705_m1
GNLY	Hs01120727_m1
GZMA	Hs00989184_m1
GZMB	Hs00188051_m1
HCK	Hs01067403_m1
HDAC4	Hs01041638_m1
HPRT1	Hs02800695_m1
ICOS	Hs00359999_m1
IDO1	Hs00984148_m1
IFI16	Hs00986757_m1
IFI35	Hs00413458_m1
IFI44	Hs00197427_m1
IFI44L	Hs00915292_m1
IFI6	Hs00242571_m1

Supplementary table 1. (Continued)

Target genes	
Gene	**TaqMan Assay**
IFIH1	Hs00223420_m1
IFIT2	Hs00533665_m1
IFIT3	Hs00155468_m1
IFIT5	Hs00202721_m1
IFITM1	Hs01652522_g1
IFITM3	Hs03057129_s1
IFNG	Hs00989291_m1
IGHD	Hs00378878_m1
IL10	Hs00961622_m1
IL12A	Hs01073447_m1
IL12B	Hs01011518_m1
IL13	Hs00174379_m1
IL15	Hs01003716_m1
IL17A	Hs00174383_m1
IL1B	Hs01555410_m1
IL1RN	Hs00893626_m1
IL2	Hs00174114_m1
IL22RA1	Hs00222035_m1
IL23A	Hs00372324_m1
IL2RA	Hs00907777_m1
IL4	Hs00174122_m1
IL5	Hs99999031_m1
IL6	Hs00174131_m1
IL7R	Hs00902334_m1
IL9	Hs00174125_m1
IRF7	Hs00185375_m1
KIF1B	Hs01114511_m1
KLRB1	Hs00174469_m1
KLRC1	Hs00242628_m1
KLRC2/3	Hs04192492_gH
KLRD1	Hs00233844_m1
LAG3	Hs99999160_m1
LEP	Hs00174877_m1
LEPR	Hs00174497_m1
LTF	Hs00914334_m1
LYN	Hs01015816_m1

Target genes	
Gene	**TaqMan Assay**
MARCO	Hs00198937_m1
MMP9	Hs00957562_m1
MRC1	Hs00267207_m1
MRC2	Hs00195862_m1
MS4A1	Hs00544819_m1
NCAM1	Hs00941830_m1
NCF1/1B/1C	Hs00165362_m1
NEDD4L	Hs00969321_m1
NLRC4	Hs00892666_m1
NLRP1	Hs00248187_m1
NLRP10	Hs00738590_m1
NLRP11	Hs00935472_m1
NLRP12	Hs00376283_m1
NLRP13	Hs00603406_m1
NLRP2	Hs01546932_m1
NLRP3	Hs00918082_m1
NLRP4	Hs00370499_m1
NLRP6	Hs00373246_m1
NLRP7	Hs00373683_m1
NOD1	Hs01036720_m1
NOD2	Hs01550753_m1
NOS2	Hs01075529_m1
OAS1	Hs00973635_m1
OAS2	Hs00942643_m1
OAS3	Hs00196324_m1
PAX5	Hs00277134_m1
PDCD1	Hs01550088_m1
PRF1	Hs00169473_m1
PTPRCv1	Hs04266413_m1
PTPRCv2	Hs00898488_m1
RAB13	Hs04400188_g1
RAB24	Hs01557556_g1
RAB33A	Hs00191243_m1
RORC	Hs01076112_m1
SCARF1	Hs01092477_m1
SEC14L1	Hs01019672_m1

Supplementary table 1. (Continued)

Target genes	
Gene	**TaqMan Assay**
SEPTIN4	Hs00365352_m1
SERPING1	Hs00163781_m1
SLAMF7	Hs00904275_m1
SOCS1	Hs00864158_g1
SPP1	Hs00959010_m1
STAT1	Hs01013996_m1
STAT2	Hs01013132_m1
TAGAP	Hs00299284_m1
TAP1	Hs00388675_m1
TAP2	Hs00241060_m1
TBC1D7	Hs00964082_m1
TBX21	Hs00894392_m1
TGFB1	Hs00998133_m1
TGFBR2	Hs00234253_m1
TIMP2	Hs00234278_m1
TLR1	Hs00413978_m1
TLR10	Hs01675179_m1
TLR2	Hs00152932_m1
TLR3	Hs00152933_m1
TLR4	Hs00152939_m1
TLR5	Hs00152825_m1
TLR6	Hs04975839_m1
TLR7	Hs00152971_m1
TLR8	Hs00152972_m1
TLR9	Hs00152973_m1
TNF	Hs00174128_m1
TNFRSF1A	Hs01042313_m1
TNFRSF1B	Hs00961750_m1
TNFRSF4	Hs00533968_m1
TNFRSF9	Hs00155512_m1
TNIP1	Hs00374581_m1
TNRSF18	Hs00188346_m1
TRAFD1	Hs00198630_m1
TWIST1	Hs00361186_m1
UCP2	Hs01075227_m1
VEGFA	Hs00900055_m1
ZNF331	Hs00367929_m1
ZNF532	Hs00539543_m1

Reference gene	
Gene	**TaqMan Assay**
GAPDH	Hs99999905_m1

Supplementary table 2. Baseline characteristics of males and females.

	Males		Females	
	Dutch Europid (*n* = 25)	**Dutch South Asian (*n* = 18)**	**Dutch Europid (*n* = 19)**	**Dutch South Asian (*n* = 27)**
Demographics				
Age, years	61.3 ± 6.1	55.7 ± 10.1*	57.4 ± 6.6	53.9 ± 10.5
Diabetes duration, years	12.1 ± 7.3	18.0 ± 11.3	8.9 ± 4.6	17.0 ± 8.9**
Clinical parameters				
Weight, kg	97.9 ± 13.8	85.1 ± 11.4**	95.8 ± 14.4	76.0 ± 11.0***
Length, cm	178.8 ± 5.3	172.9 ± 7.2**	165.9 ± 7.2	159.0 ± 4.1***
BMI, kg/m^2	30.5 ± 3.2	28.5 ± 4.0	34.7 ± 3.7	30.0 ± 4.0***
Waist-height ratio	0.61 ± 0.04	0.59 ± 0.05	0.67 ± 0.05	0.63 ± 0.07*
Fat percentage, %	29.7 ± 3.7	27.6 ± 4.3	46.4 ± 5.1	43.2 ± 5.5*
VAT/SAT ratio	0.8 ± 0.3	0.7 ± 0.3	0.4 ± 0.2	0.5 ± 0.3
HbA1c, mmol/mol	68.2 ± 10.8	68.9 ± 12.2	63.4 ± 10.9	66.9 ± 11.1
HbA1c, %	8.4 ± 1.0	8.5 ± 1.1	8.0 ± 1.0	8.3 ± 1.0
Cholesterol, mmol/L	4.7 ± 1.0	4.1 ± 1.0*	5.0 ± 1.0	4.4 ± 0.9*
HDL-C, mmol/L	1.2 ± 0.3	1.1 ± 0.3	1.3 ± 0.3	1.3 ± 0.3
LDL-C, mmol/L	2.5 ± 0.9	1.9 ± 0.9*	2.7 ± 0.9	2.3 ± 0.8
CRP, mmol/L	2.2 ± 1.6	1.8 ± 1.9	4.3 ± 4.4	4.9 ± 4.7
Diabetic complications/comorbidity				
Diabetic retinopathy, n, %	4, 16%	11, 61%**	0, 0%	12, 44%**
Diabetic nephropathy, n, %	9, 36%	6, 33%	2, 11%	4, 15%
Diabetic neuropathy, n, %	10, 40%	5, 28%	5, 26%	7, 26%
Macrovascular disease, n, %	2, 8%	7, 39%*	0, 0%	5, 19%*
Concomitant medication use				
Metformin, mg/day	2032 ± 547	1826 ± 710	2066 ± 612	1700 ± 633
Sulfonylurea, n, %	6, 24%	2, 11%	7, 37%	6, 22%
Insulin use, n, %	15, 60%	13, 72%	13, 68%	21, 78%
Statin, n, %	18, 72%	16, 89%	16, 84%	16, 59%
Anti-hypertensive drug, n, %	22, 88%	16, 89%	14, 74%	18, 67%

Data are presented as mean ± standard deviation, unless specified otherwise. P values indicate differences between Dutch Europid versus Dutch South Asian patients. *P<0.05, **P<0.01, ***P<0.001. BMI, body mass index; CRP, C-reactive protein, HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.