INFLAMED FAT: immune modulation of adipose tissue and lipid metabolism

Dam, A.D. van; Dam A.D. van

Chapter 4

South Asians have lower expression of interferon signaling genes in white adipose tissue and skeletal muscle compared to white Caucasians

Andrea D. van Dam, Mark J.W. Hanssen, Robin van Eenige, Edwin Quinten, Hetty C. Sips, Cindy J.M. Hülsman, Ingrid M. Jazet, Wouter D. van Marken Lichtenbelt, Tom H.M. Ottenhoff, Mariëlle C. Haks, Patrick C.N. Rensen, Mariëtte R. Boon

Submitted

ABSTRACT

South Asians have a higher risk of developing type 2 diabetes compared to white Caucasians. Since inflammation plays an important role in type 2 diabetes development, we assessed inflammatory state in South Asians compared to white Caucasians. We assessed transcriptomic levels of 144 inflammatory, immune-regulating and immune cell subset markers in blood, skeletal muscle and white adipose tissue (WAT) of overweight pre-diabetic Dutch South Asian and matched white Caucasian men using a focused multiplex gene expression profiling technique. In South Asians, expression of especially interferon signaling genes was lower, both in muscle (*IFIT3*, *IFI44*; -45-51%) and in WAT (*IFI35*, *IFI44*, *IFIT2*, *IFIT3*, *IFIT5*, *OAS1*, *STAT1*; -13-40%). Of note, *IL5* expression was also lower in WAT (-66%). In conclusion, South Asians have lower expression levels of interferon signaling genes in skeletal muscle and WAT. Future studies should investigate the relevance of interferon signaling for type 2 diabetes development.

INTRODUCTION

South Asians, who originate from the Indian subcontinent, make up 20% of the world population and have a higher risk of developing type 2 diabetes when compared to white Caucasians (1). Albeit central obesity and insulin resistance are more prevalent in South Asians than in white Caucasians (1), these predisposing classical risk factors cannot fully explain their excess risk of developing type 2 diabetes. Despite the clear link between inflammation and the pathogenesis of type 2 diabetes (2), comprehensive data on the inflammatory state in South Asians are lacking. Therefore, the aim of the current study was to compare transcriptomic levels of a large panel of inflammatory, immune-regulating and immune cell subset markers in blood, skeletal muscle and WAT of overweight, pre-diabetic South Asian and matched white Caucasian men.

MATERIALS AND METHODS

Participants

Ten Dutch overweight (body mass index (BMI) between 25 and 35 kg/m²), pre-diabetic middle-aged (35-55 y) South Asian males and ten BMI- and age-matched Dutch white Caucasian males were included in this study. Pre-diabetes was defined as fasting plasma glucose levels between 5.6 and 6.9 mmol/L or plasma glucose levels between 7.8 and 11.1 mmol/L at 2 hours after an oral glucose tolerance test. Ethnicity was specified as having four grandparents of white Caucasian or South Asian origin. Exclusion criteria included type 2 diabetes, smoking, vigorous exercise, liver or kidney dysfunction, hypo- or hyperthyroidism, uncontrolled hypertension and the use of beta-blockers. Three South Asians used antihypertensive medication before and during the study. The study was originally designed to assess the effect of L-arginine on energy expenditure and mitochondrial function, for which a part of this study will be published elsewhere (Hanssen & Boon).

Study approval

This study was approved by the Ethics Committee of Maastricht University Medical Center and the Leiden University Medical Center (The Netherlands). Procedures were conducted according to the principles of the Declaration of Helsinki. All participants provided written informed consent. Trial registration number: NCT02291458 (Hanssen & Boon).

Study design

Subjects were given placebo or L-arginine (Argimax®, Hankintatukku Oy) for six weeks (9 g/day, divided over three quantities per day after breakfast, lunch and dinner) in a randomized double-blind cross-over design with a four-week washout period in between. Subjects were instructed to abstain from heavy physical exercise during the last 2 days of the treatment period, and standardized evening meals were consumed before the experimental days. Each intervention period was followed by 2 study days. On the first study day, a 4h-fasted

blood sample was drawn and resting energy expenditure was measured during 45 min at thermoneutrality (*i.e.* 29.9±0.1°C) and during 90 min of cold exposure (*i.e.* 23.9±0.4°C) via a face-mask connected to an indirect calorimeter (EZcal, IDEE). Furthermore, a cold-induced [^{18}F]fluorodeoxyglucose (FDG) PET-CT scan was conducted to assess brown adipose tissue (BAT) volume and activity. On the second day, skeletal muscle biopsies (approx. 75-100 mg) were taken from the *musculus vastus lateralis* and subcutaneous white adipose tissue (WAT) biopsies were taken from the umbilical region under localized anesthesia in the morning after an overnight fast. Dual-energy X-ray absorptiometry (DEXA) was performed to assess body composition. The study was performed between November 2014 and October 2015 in Maastricht, The Netherlands (Hanssen & Boon). From all subjects, measurements after placebo treatment were used to study the transcriptomic levels of inflammatory, immune-regulating and immune cell subset markers in South Asians *vs.* white Caucasians.

RNA isolation and dual-color RT-MLPA assay

Muscle and WAT biopsies were homogenized in 1 mL TriPure RNA Isolation reagent (Roche, The Netherlands) and RNA was extracted according to manufacturer's instructions. Whole blood was collected in venipuncture PAXgene collection tubes and total RNA was extracted and purified using the PAXgene Blood miRNA kit (PreAnalytix). A dual-color Reverse Transcriptase Multiplex Ligation-dependent Probe Amplification (dcRT-MLPA) assay (3, 4) was performed on blood, WAT and skeletal muscle tissue. Briefly, for each target-specific sequence, a specific RT primer was designed located immediately downstream of the left and right hand half-probe target sequence. Following reverse transcription, left and right hand half-probes were hybridized to the cDNA at 60°C overnight. Annealed half-probes were ligated and subsequently amplified by PCR. PCR amplification products were 1:10 diluted in HiDi formamide-containing 400HD ROX size standard and analysed on an Applied Biosystems 3730 capillary sequencer in GeneScan mode (Baseclear). Trace data were analyzed using the GeneMapper 5.0 software package (Applied Biosystems). The areas of each assigned peak (in arbitrary units) were exported for further analysis in Microsoft Excel. Data were normalized to *GAPDH* and signals below the threshold value for noise cutoff in GeneMapper (log2 transformed peak area 7.64) were assigned the threshold value for noise cut-off (5, 6).

Statistical analysis

All data are expressed as means ± SEM. If some but not all subjects within a group showed an expression level below the detection limit, the value corresponding to the limit of detection divided by 2 was used for calculation of the mean and statistical analysis. If data were normally distributed as assessed by a Shapiro-Wilk test, log-transformed data were compared with a two-tailed unpaired Student's t-test and if not normally distributed with a Mann-Whitney U test. Correlations were analysed by linear regression analysis. IBM SPSS Statistics 23.0 was used for analyses and differences and correlations were considered statistically significant if $p<0.05$.

RESULTS

Clinical characteristics

White Caucasians and South Asians had comparable age, BMI and fasting glucose, though South Asians tended to be shorter (-2.8%; p=0.06) (**Table 1**).

Markers of inflammation in blood

Transcriptomic profiles of immune markers in blood are listed in **Supplementary Table 1**. Several markers were expressed higher in South Asians, including *GNLY* (+79%), *NOD2* (+40%), *IL2RA* (+33%), *CCL5* (+32%), *NLRP3* (+27%) and *PRF1* (+23%). In contrast, expression of *CCL19* (-47%), *IL6* (-30%), *FPR1* (-29%), *DSE* (-24%), *CXCL10* (-22%) and *TGFBR2* (-12%) was lower in the blood of South Asians.

Markers of inflammation in muscle and WAT

In muscle, the expression of many immune markers was below the detection limit (**Supplementary Table 1**). Nevertheless, expression levels of individual genes like *LAG3* (+79%), *TNIP1* (+49%), *IL23A* (+39%) and *NLRC4* (+32%) were higher in South Asians. The two genes most significantly lower expressed in South Asians were interferon signaling genes *IFI44* (-51%) and *IFIT3* (-45%) (**Fig. 1A**). In addition, *CTLA4* (-44%), *CXCL10* (-39%), *NCAM1* (-38%), *TNFRSF1A* (-38%), *IL13* (-36%), *SEC14L1* (-33%) and *TGFB1* (-14%) were lower expressed.

Table 1. Baseline metabolic characteristics and body composition after 6 weeks placebo treatment of white Caucasians and South Asians. *Body composition was determined by DEXA. BMI, body mass index; OGTT, oral glucose tolerance test. Data are presented as mean ± SEM.*

	White Caucasians n=10	South Asians n=10	P-value
Age (years)	47.5 ± 2.0	46.5 ± 2.3	0.744
Height (m)	1.81 ± 0.02	1.76 ± 0.02	0.061
Weight (kg)	99.9 ± 4.0	93.0 ± 3.8	0.223
BMI (kg/m²)	30.7 ± 1.2	30.1 ± 1.1	0.723
Fasting glucose (mmol/L)	5.7 ± 0.2	5.6 ± 0.2	0.939
OGTT2h glucose (mmol/L)	7.4 ± 0.6	6.6 ± 0.5	0.282
HbA1c (%; mmol/mol)	5.4 ± 0.1; 36.0 ± 0.9	5.7 ± 0.1; 38.3 ± 1.3	0.149
Total cholesterol (mmol/L)	5.60 ± 0.29	5.51 ± 0.24	0.819
TG (mmol/L)	1.72 ± 0.20	1.95 ± 0.41	0.628
Fat percentage (%)	30.1 ± 1.0	31.2 ± 1.3	0.540
Lean mass percentage (%)	67.6 ± 1.0	66.3 ± 1.3	0.458
Trunk/limb fat mass ratio	1.2 ± 0.1	1.2 ± 0.1	0.698
Visceral adipose tissue mass (g)	714 ± 79	689 ± 36	0.771
Visceral adipose tissue volume (cm³)	772 ± 86	745 ± 39	0.768

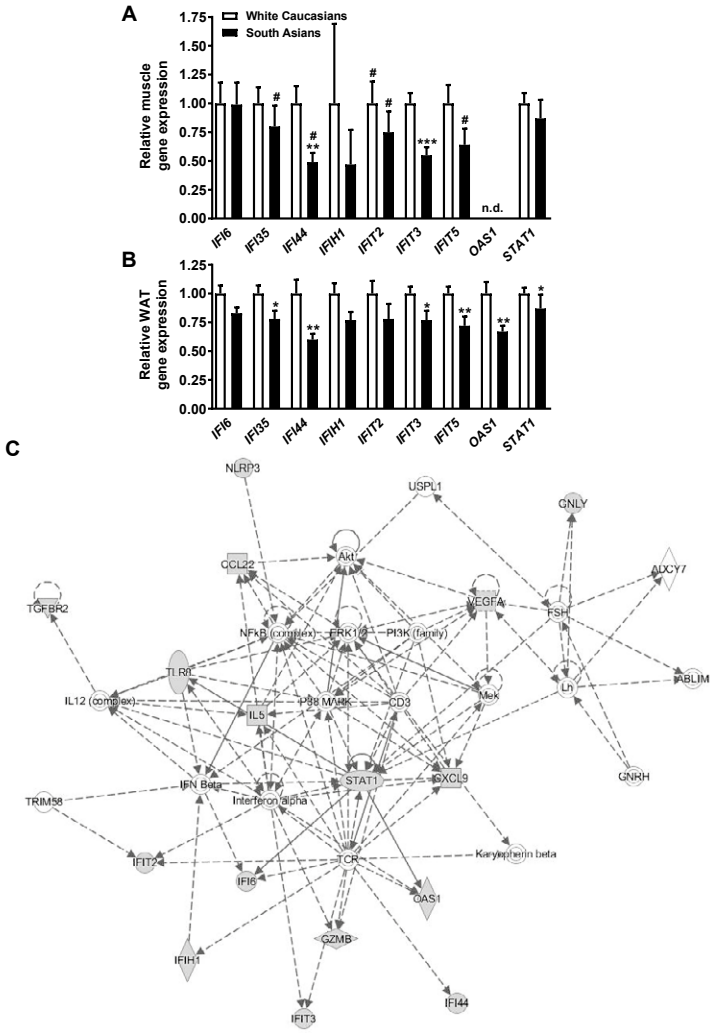

Figure 1. Interferon signaling gene expression is lower in muscle and white adipose tissue of South Asians compared to white Caucasians. *Relative interferon signaling gene expression in (A) muscle and (B) white adipose tissue of white Caucasians and South Asians. N.d., not detectable; WAT, white adipose tissue. Data are presented as mean ± SEM. *p<0.05, **p<0.01, ***p<0.001 compared to white Caucasians, #Gene expression of 4 or more individuals was not detectable. (C) Ingenuity Pathway Analysis (IPA) was performed on the list of genes with a (tendency for; p<0.1) different expression between South Asians and white Caucasians, which are marked with grey symbols in the network. Genes in uncoloured symbols were not analysed or identified as differentially expressed in our experiment and were integrated into the computationally generated network based on the evidence stored in the IPA knowledge memory indicating relevance to this network. The top canonical pathway was interferon signaling. Gene and gene relationship symbols: square, cytokine; vertical diamond, enzyme; horizontal diamond, peptidase; double circle, group or complex; dashed square, growth factor; triangle, kinase; horizontal oval, transcription factor; vertical oval, transmembrane receptor; single circle, other; arrow, acts on; continuous line, direct interaction; dashed line, indirect interaction.*

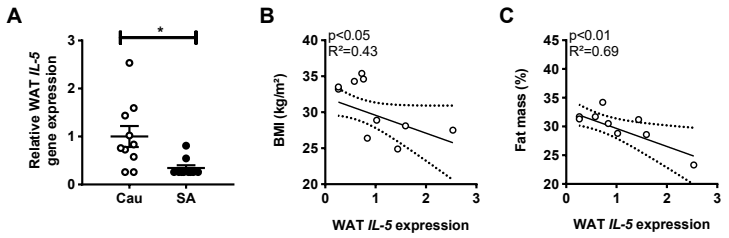

Figure 2. *IL5* gene expression is lower in white adipose tissue of South Asians compared to white Caucasians. *(A) Relative IL5 mRNA expression in white adipose tissue (WAT) of white Caucasians and South Asians. Data are presented as mean ± SEM. *p<0.05 compared to white Caucasians. Correlation of (B) body mass index (BMI) and (C) fat mass with IL5 mRNA expression in WAT of white Caucasians. WAT, white adipose tissue; WC, white Caucasians; SA, South Asians; BMI, body mass index.*

In WAT, several immune markers having a diverse range of immunological functions were differentially expressed between ethnicities including *GNLY* (+58%), *BPI* (-46%), *CCL22* (-34%), *CXCL9* (-28%) and *TGFBR2* (-17%) (**Supplementary Table 1**). Interestingly, in line with skeletal muscle, out of the 12 genes with significantly lower expression in South Asians, 7 were interferon signaling genes, including *IFI35* (-22%), *IFI44* (-40%), *IFIT2* (-22%), *IFIT3* (-23%), *IFIT5* (-28%), *OAS1* (-33%) and *STAT1* (-13%). *IFI6* (-17%) and *IFIH1* (-23%) expression also tended to be reduced (**Fig. 1B**).

To identify the signaling pathways that are differently regulated in WAT, genes with a tendency for different expression between South Asians and white Caucasians ($p<0.1$) were fed into Ingenuity Pathway Analysis (IPA). The top canonical pathway enriched with differentially expressed genes between the two ethnicities was interferon signaling (**Fig. 1C**), confirming our analysis of individual genes that are differentially expressed in WAT of South Asians and white Caucasians.

Of note, the most differently expressed gene in WAT of South Asians was *IL5* (-66%), which was mainly due to a lack of *IL5* expression in most South Asians (**Fig. 2A**). Remarkably, *IL5* expression in WAT of only white Caucasians (as expression was undetectable in South Asians), negatively correlated with BMI ($R^2=0.43$, $p<0.05$) and fat mass ($R^2=0.69$, $p<0.01$; **Fig. 2B-C**).

DISCUSSION

We demonstrate that many immune markers involved in a range of immunological functions in blood, muscle and WAT are differentially expressed in South Asians compared to white Caucasians. The most pronounced differences included lower expression of interferon signaling genes (skeletal muscle and WAT) and *IL5* (WAT) in South Asians.

Despite the high risk of type 2 diabetes in South Asians and the clear link between inflammation and type 2 diabetes, inflammation in South Asians has not been extensively studied. South Asians have higher plasma CRP levels compared to white Caucasians, suggesting a more pro-inflammatory state. Moreover, South Asians generally have more visceral adipose tissue, which is an important source of pro-inflammatory cytokines (7). One previous study observed similar CD68 protein content but lower CCL2 levels in muscle of South Asians compared to white Caucasians (8). Although we did not determine *CD68* expression, many macrophage markers including *CCL2* were undetectable in muscle of both ethnicities.

The most striking finding of the current study was that in both WAT and muscle, interferon signaling genes were consistently lower expressed in South Asians. Ingenuity pathway analysis corroborated the finding that the anti-inflammatory type 1 interferon signaling pathways, *i.e.* interferon α and β, were lower expressed in South Asians. Interestingly, adipose tissue-specific knockout of *interferon (α+β) receptor 1* in mice deteriorates high-fat diet induced weight gain, insulin resistance and glucose intolerance (9). On the other hand, IFNβ1 induces cellular glucose uptake via the PI3K/Akt pathway (10) and overexpression of *Ifnβ1* suppresses adipose tissue inflammation and protects against diet-induced obesity and glucose intolerance (11). Together, this indicates that impaired interferon signaling in South Asians may, at least partly, cause their predisposition for development of obesity as well as type 2 diabetes.

In addition, *IL5* expression was markedly lower expressed in WAT of South Asians. *IL5* is produced by group 2 innate lymphoid cells (ILC2s) and is essential for mobilizing eosinophils in the bone marrow and regulating homing and migration of eosinophils into tissues. *IL5* transgenic mice are hypereosinophilic and have reduced fat mass and improved glucose tolerance (12), which is in accordance with our data showing that *IL5* expression in adipose tissue of white Caucasians negatively correlates with fat mass. ILC2s also promote browning of WAT (13) and may thereby enhance energy expenditure and protect against obesity and associated disorders. Based on the findings in rodents, we speculate that low *IL5* expression in South Asians may be accompanied by low adipose tissue eosinophils, reduced browning and its unfavorable consequences such as higher fat mass and reduced glucose tolerance.

Limitations of our study are the small sample size and the placebo treatment that participants received in the context of the L-arginine treatment this trial was originally designed for. As we only assessed gene expression levels, further research into the immune system of South Asians would also benefit from performing analysis of protein levels and flow cytometry. Strengths of our study are the extensiveness of the panel of immune

markers measured, not only in blood, but also in two important metabolic tissues within the same individuals. Furthermore, the individuals of different ethnicities were well-matched for metabolic parameters that could interfere with inflammation, such as age and BMI.

In conclusion, the immune system in blood, muscle and WAT is differently primed in South Asians compared to white Caucasians, with a consistent lower expression of interferon signaling genes in metabolic tissues of South Asians as the most prominent feature. Whether interferon signaling in metabolic tissues directly influences glucose metabolism in humans and whether this could be a target to treat insulin resistance or reduce the risk of type 2 diabetes in South Asians is an interesting field of future investigation.

ACKNOWLEDGEMENTS

We thank Chris van der Bent and Trea Streefland (Dept. of Medicine, Div. of Endocrinology, LUMC, Leiden, The Netherlands) for their excellent technical assistance.

FUNDING

This work was supported by a grant of the Rembrandt Institute of Cardiovascular Science (RICS) and a Rubicon grant of the Netherlands Organization for Scientific Research to Mariëtte R. Boon. Mariëtte R. Boon is also supported by the Dutch Diabetes Foundation (grant 2015.81.1808). Patrick C.N. Rensen is an Established Investigator of the Netherlands Heart Foundation (grant 2009T038).

REFERENCES

1. Raji A, Seely EW, Arky RA, Simonson DC: Body fat distribution and insulin resistance in healthy Asian Indians and Caucasians. The Journal of clinical endocrinology and metabolism 2001;86:5366-5371
2. Boon MR, Bakker LE, van der Linden RA, van Ouwerkerk AF, de Goeje PL, Counotte J, Jazet IM, Rensen PC: High prevalence of cardiovascular disease in South Asians: Central role for brown adipose tissue? Critical reviews in clinical laboratory sciences 2015;52:150-157
3. Haks MC, Goeman JJ, Magis-Escurra C, Ottenhoff TH: Focused human gene expression profiling using dual-color reverse transcriptase multiplex ligation-dependent probe amplification. Vaccine 2015;33:5282-5288
4. Joosten SA, Goeman JJ, Sutherland JS, Opmeer L, de Boer KG, Jacobsen M, Kaufmann SH, Finos L, Magis-Escurra C, Ota MO, Ottenhoff TH, Haks MC: Identification of biomarkers for tuberculosis disease using a novel dual-color RT-MLPA assay. Genes and immunity 2012;13:71-82
5. Boon MR, Bakker LE, Haks MC, Quinten E, Schaart G, Van Beek L, Wang Y, Van Schinkel L, Van Harmelen V, Meinders AE, Ottenhoff TH, Van Dijk KW, Guigas B, Jazet IM, Rensen PC: Short-term high-fat diet increases macrophage markers in skeletal muscle accompanied by impaired insulin signalling in healthy male subjects. Clinical science (London, England : 1979) 2015;128:143-151
6. van der Lans AA, Boon MR, Haks MC, Quinten E, Schaart G, Ottenhoff TH, van Marken Lichtenbelt WD: Cold acclimation affects immune composition in skeletal muscle of healthy lean subjects. Physiological reports 2015;3:e12394
7. Chambers JC, Eda S, Bassett P, Karim Y, Thompson SG, Gallimore JR, Pepys MB, Kooner JS: C-reactive protein, insulin resistance, central obesity, and coronary heart disease risk in Indian Asians from the United Kingdom compared with European whites. Circulation 2001;104:145-150
8. Samaan MC, Anand SS, Sharma AM, Bonner A, Beyene J, Samjoo I, Tarnopolsky MA: Adiposity and immune-muscle crosstalk in South Asians & Europeans: A cross-sectional study. Scientific reports 2015;5:14521
9. Wieser V, Adolph TE, Grander C, Grabherr F, Enrich B, Moser P, Moschen AR, Kaser S, Tilg H: Adipose type I interferon signalling protects against metabolic dysfunction. Gut 2016; in press
10. Burke JD, Platanias LC, Fish EN: Beta interferon regulation of glucose metabolism is PI3K/Akt dependent and important for antiviral activity against coxsackievirus B3. Journal of virology 2014;88:3485-3495
11. Alsaggar M, Mills M, Liu D: Interferon beta overexpression attenuates adipose tissue inflammation and high-fat diet-induced obesity and maintains glucose homeostasis. Gene therapy 2016;24:60-66
12. Wu D, Molofsky AB, Liang HE, Ricardo-Gonzalez RR, Jouihan HA, Bando JK, Chawla A, Locksley RM: Eosinophils sustain adipose alternatively activated macrophages associated with glucose homeostasis. Science (New York, NY) 2011;332:243-247
13. Brestoff JR, Kim BS, Saenz SA, Stine RR, Monticelli LA, Sonnenberg GF, Thome JJ, Farber DL, Lutfy K, Seale P, Artis D: Group 2 innate lymphoid cells promote beiging of white adipose tissue and limit obesity. Nature 2015;519:242-246

SUPPLEMENTARY APPENDIX

Supplementary Table 1. Relative gene expression levels of immune markers in blood, skeletal muscle and white adipose tissue (WAT) of white Caucasians and South Asians. *N.d., not detectable. Data are presented as mean ± SEM, n=10 per group. *p<0.05, **p<0.01, ***p<0.001 compared to white Caucasians, #Gene expression of 4 or more individuals was not detectable.*

	Blood		Muscle		WAT	
Gene	**White Caucasians**	**South Asians**	**White Caucasians**	**South Asians**	**White Caucasians**	**South Asians**
Immune cell subset markers						
BLR1	1.00 ± 0.10	1.20 ± 0.11	1.00 ± 0.15#	0.87 ± 0.11#	1.00 ± 0.15	1.12 ± 0.18
CD19	1.00 ± 0.10	1.09 ± 0.12	n.d.#	n.d.#	n.d.#	n.d.#
NCAM1	1.00 ± 0.20	1.27 ± 0.17	1.00 ± 0.13	**0.62 ± 0.10#***	1.00 ± 0.19	0.86 ± 0.17
T cell subsets						
CD3E	1.00 ± 0.13	1.22 ± 0.07	n.d.#	n.d.#	1.00 ± 0.20#	1.38 ± 0.18
CD4	1.00 ± 0.06	0.97 ± 0.04	n.d.#	n.d.#	1.00 ± 0.07	0.89 ± 0.05
CD8A	1.00 ± 0.26	1.38 ± 0.24	n.d.#	n.d.#	n.d.#	n.d.#
CCR7	1.00 ± 0.16	1.14 ± 0.09	1.00 ± 0.17#	1.80 ± 0.38	1.00 ± 0.00#	1.14 ± 0.14#
PTPRCv1	1.00 ± 0.09	1.17 ± 0.08	n.d.#	n.d.#	1.00 ± 0.21#	0.78 ± 0.16#
PTPRCv2	1.00 ± 0.11	1.08 ± 0.06	n.d.#	n.d.#	1.00 ± 0.14	0.92 ± 0.10
AIRE	1.00 ± 0.13	0.80 ± 0.09	n.d.#	n.d.#	1.00 ± 0.11	1.06 ± 0.09
Th1 response						
CXCL10	1.00 ± 0.10	**0.78 ± 0.06***	1.00 ± 0.11	**0.61 ± 0.08#***	1.00 ± 0.10	0.93 ± 0.13
IFNG	n.d.#	n.d.#	n.d.#	n.d.#	n.d.#	n.d.#
IL1B	1.00 ± 0.10	0.88 ± 0.04	n.d.#	n.d.#	1.00 ± 0.17#	1.29 ± 0.19#
IL2	n.d.#	n.d.#	n.d.#	n.d.#	n.d.#	n.d.#
IL15	n.d.#	n.d.#	n.d.#	n.d.#	1.00 ± 0.13#	0.81 ± 0.00#
TBX21	1.00 ± 0.18#	0.95 ± 0.16#	n.d.#	n.d.#	n.d.#	n.d.#
TNF	1.00 ± 0.07	0.94 ± 0.04	1.00 ± 0.07	1.11 ± 0.10	1.00 ± 0.05	0.91 ± 0.07
Th2 response						
GATA3	1.00 ± 0.11	1.23 ± 0.08	n.d.#	n.d.#	1.00 ± 0.13#	1.30 ± 0.18#
IL4	1.00 ± 0.41#	0.38 ± 0.00#	n.d.#	n.d.#	1.00 ± 0.16#	0.77 ± 0.00#
IL4d2	1.00 ± 0.18	0.98 ± 0.13	n.d.#	n.d.#	1.00 ± 0.19#	0.94 ± 0.13
IL5	1.00 ± 0.16	0.80 ± 0.05	n.d.#	n.d.#	1.00 ± 0.22	**0.34 ± 0.06#****
IL6	1.00 ± 0.09	**0.70 ± 0.05***	1.00 ± 0.10#	1.04 ± 0.14#	1.00 ± 0.16	0.98 ± 0.09
IL9	1.00 ± 0.12	0.84 ± 0.07	n.d.#	n.d.#	1.00 ± 0.09	1.04 ± 0.10
IL13	n.d.#	n.d.#	1.00 ± 0.09	**0.64 ± 0.10***	1.00 ± 0.00#	1.23 ± 0.23#

	Blood		Muscle		WAT	
Gene	**White Caucasians**	**South Asians**	**White Caucasians**	**South Asians**	**White Caucasians**	**South Asians**
Th17 response						
IL17A	n.d.#	n.d.#	n.d.#	n.d.#	1.00 ± 0.00#	2.21 ± 0.99#
RORC	1.00 ± 0.26#	0.74 ± 0.00#	1.00 ± 0.14	0.80 ± 0.06	n.d.#	n.d.#
NEDD4L	1.00 ± 0.06	1.19 ± 0.09	1.00 ± 0.14	0.91 ± 0.08	1.00 ± 0.07	0.95 ± 0.08
IL22RA1	1.00 ± 0.11	1.10 ± 0.05	n.d.#	n.d.#	n.d.#	n.d.#
Treg markers						
CTLA4	1.00 ± 0.06	1.02 ± 0.04	1.00 ± 0.14	**0.56 ± 0.22***	1.00 ± 0.08	1.04 ± 0.08
FOXP3	1.00 ± 0.14	1.15 ± 0.07	n.d.#	n.d.#	1.00 ± 0.15	1.04 ± 0.12
IL10	1.00 ± 0.12	1.12 ± 0.08	n.d.#	n.d.#	1.00 ± 0.11	0.94 ± 0.08
IL2RA	1.00 ± 0.08	**1.33 ± 0.08****	n.d.#	n.d.#	1.00 ± 0.22#	1.06 ± 0.19#
IL7R	1.00 ± 0.11	1.11 ± 0.06	n.d.#	n.d.#	1.00 ± 0.11	0.96 ± 0.08
LAG3	1.00 ± 0.00#	1.33 ± 0.33#	1.00 ± 0.33	**1.79 ± 0.24***	n.d.#	n.d.#
TGFB1	1.00 ± 0.05	1.00 ± 0.03	1.00 ± 0.04	**0.86 ± 0.06***	1.00 ± 0.04	0.95 ± 0.04
TNFRSF18	1.00 ± 0.08	0.90 ± 0.14	n.d.#	n.d.#	1.00 ± 0.19#	1.11 ± 0.17#
Cytotoxicity markers						
GNLY	1.00 ± 0.17	**1.79 ± 0.19****	1.00 ± 0.00#	1.37 ± 0.19#	1.00 ± 0.23	**1.58 ± 0.23***
GZMA	1.00 ± 0.16	1.08 ± 0.13	n.d.#	n.d.#	1.00 ± 0.07	0.97 ± 0.09
GZMB	1.00 ± 0.21	1.32 ± 0.11	n.d.#	n.d.#	1.00 ± 0.13	1.37 ± 0.15
PRF1	1.00 ± 0.19	**1.23 ± 0.08***	n.d.#	n.d.#	1.00 ± 0.15	1.04 ± 0.18
Antimicrobial activity						
LTF	1.00 ± 0.22	0.58 ± 0.13	n.d.#	n.d.#	1.00 ± 0.40#	0.69 ± 0.25#
Macrophage markers						
CD14	1.00 ± 0.07	0.84 ± 0.04	1.00 ± 0.15	0.96 ± 0.25	1.00 ± 0.07	0.87 ± 0.08
CD163	1.00 ± 0.08	0.80 ± 0.04	1.00 ± 0.23	0.72 ± 0.33#	1.00 ± 0.07	0.83 ± 0.08
CD209	1.00 ± 0.08	0.99 ± 0.05	1.00 ± 0.15	1.10 ± 0.07	1.00 ± 0.09	0.99 ± 0.07
CCL2	1.00 ± 0.19#	0.92 ± 0.18#	n.d.#	n.d.#	1.00 ± 0.12	1.17 ± 0.24
CCL3	1.00 ± 0.08	1.07 ± 0.09	1.00 ± 0.51	0.38 ± 0.10	1.00 ± 0.07	1.23 ± 0.27
CCL4	1.00 ± 0.07	0.95 ± 0.04	n.d.#	n.d.#	1.00 ± 0.03	0.94 ± 0.04
CCL5	1.00 ± 0.11	**1.32 ± 0.09***	1.00 ± 0.17#	0.96 ± 0.14#	1.00 ± 0.11	1.07 ± 0.12
CCL22	1.00 ± 0.15	0.87 ± 0.05	n.d.#	n.d.#	1.00 ± 0.08	**0.66 ± 0.07****
CXCL13	1.00 ± 0.13	0.75 ± 0.22#	n.d.#	n.d.#	1.00 ± 0.20#	1.27 ± 0.27
IL12A	1.00 ± 0.15#	0.92 ± 0.14#	1.00 ± 0.25#	0.75 ± 0.00#	1.00 ± 0.16#	1.04 ± 0.20#
IL12B	1.00 ± 0.11	0.96 ± 0.05	1.00 ± 0.00#	1.14 ± 0.14#	1.00 ± 0.09	1.03 ± 0.10
IL23A	1.00 ± 0.03	1.05 ± 0.03	1.00 ± 0.09	**1.39 ± 0.10****	1.00 ± 0.04	1.00 ± 0.03
Scavenger receptors						
MARCO	1.00 ± 0.20	0.48 ± 0.14#	n.d.#	n.d.#	1.00 ± 0.43#	0.50 ± 0.12#

	Blood		Muscle		WAT	
Gene	White Caucasians	South Asians	White Caucasians	South Asians	White Caucasians	South Asians
Pattern recognition receptors						
CLEC7A	1.00 ± 0.09	0.86 ± 0.07	n.d.#	n.d.#	1.00 ± 0.15	0.79 ± 0.07
MRC1	n.d.#	n.d.#	n.d.#	n.d.#	1.00 ± 0.09	0.91 ± 0.09
MRC2	n.d.#	n.d.#	n.d.#	n.d.#	1.00 ± 0.11	1.00 ± 0.09
NOD1	1.00 ± 0.11	1.22 ± 0.09	n.d.#	n.d.#	1.00 ± 0.06	0.94 ± 0.06
NOD2	1.00 ± 0.14	**1.40 ± 0.06****	n.d.#	n.d.#	n.d.#	n.d.#
TLR1	1.00 ± 0.10	0.98 ± 0.07	n.d.#	n.d.#	1.00 ± 0.09	0.90 ± 0.10
TLR2	1.00 ± 0.12	0.91 ± 0.05	n.d.#	n.d.#	n.d.#	n.d.#
TLR3	1.00 ± 0.22#	0.90 ± 0.13	n.d.#	n.d.#	1.00 ± 0.04	0.98 ± 0.07
TLR4	1.00 ± 0.09	0.95 ± 0.06	n.d.#	n.d.#	1.00 ± 0.05	0.95 ± 0.05
TLR5	1.00 ± 0.14	0.85 ± 0.08	1.00 ± 0.47#	0.97 ± 0.16	1.00 ± 0.19#	2.68 ± 1.66#
TLR6	1.00 ± 0.09	0.88 ± 0.06	n.d.#	n.d.#	1.00 ± 0.12	1.19 ± 0.12
TLR7	1.00 ± 0.11	1.11 ± 0.07	1.00 ± 0.18#	0.96 ± 0.15#	1.00 ± 0.06	1.06 ± 0.09
TLR8	1.00 ± 0.20	1.06 ± 0.18	n.d.#	n.d.#	1.00 ± 0.25#	1.36 ± 0.16
TLR9	1.00 ± 0.51#	0.37 ± 0.30#	1.00 ± 0.10	1.21 ± 0.09	1.00 ± 0.11	0.96 ± 0.09
TLR10	n.d.#	n.d.#	n.d.#	n.d.#	n.d.#	n.d.#
Inflammasome components						
NLRC4	1.00 ± 0.07	0.99 ± 0.04	1.00 ± 0.10	**1.32 ± 0.10***	1.00 ± 0.05	1.07 ± 0.08
NLRP1	1.00 ± 0.10	1.06 ± 0.06	1.00 ± 0.00#	1.54 ± 0.23#	1.00 ± 0.06	0.94 ± 0.08
NLRP2	1.00 ± 0.16	0.90 ± 0.07	1.00 ± 0.16	1.22 ± 0.12	1.00 ± 0.09	1.01 ± 0.12
NLRP3	1.00 ± 0.07	**1.27 ± 0.06***	n.d.#	n.d.#	1.00 ± 0.14#	0.63 ± 0.09#
NLRP4	1.00 ± 0.10	0.76 ± 0.10	n.d.#	n.d.#	1.00 ± 0.10	1.05 ± 0.13
NLRP6	1.00 ± 0.13#	0.90 ± 0.09#	n.d.#	n.d.#	n.d.#	n.d.#
NLRP7	n.d.#	n.d.#	n.d.#	n.d.#	n.d.#	n.d.#
NLRP10	n.d.#	n.d.#	n.d.#	n.d.#	n.d.#	n.d.#
NLRP11	n.d.#	n.d.#	n.d.#	n.d.#	n.d.#	n.d.#
NLRP12	1.00 ± 0.10	0.92 ± 0.05	n.d.#	n.d.#	n.d.#	n.d.#
NLRP13	1.00 ± 0.30	1.08 ± 0.47	n.d.#	n.d.#	1.00 ± 0.08	0.80 ± 0.15#

	Blood		Muscle		WAT	
Gene	White Caucasians	South Asians	White Caucasians	South Asians	White Caucasians	South Asians
IFN signaling genes						
CD274	1.00 ± 0.09	1.03 ± 0.11	1.00 ± 0.25#	1.46 ± 0.23	1.00 ± 0.13#	1.06 ± 0.15#
FCGR1A	1.00 ± 0.15	1.37 ± 0.51	n.d.#	n.d.#	1.00 ± 0.16#	1.33 ± 0.24#
GBP1	1.00 ± 0.26	1.12 ± 0.20	n.d.#	n.d.#	1.00 ± 0.19#	0.98 ± 0.31#
GBP2	1.00 ± 0.11	1.10 ± 0.11	1.00 ± 0.14	0.90 ± 0.18	1.00 ± 0.08	0.90 ± 0.07
GBP5	1.00 ± 0.19	1.17 ± 0.17	n.d.#	n.d.#	1.00 ± 0.13	0.97 ± 0.14
IFI6	1.00 ± 0.42	0.72 ± 0.09	1.00 ± 0.18	0.99 ± 0.19	1.00 ± 0.07	0.83 ± 0.05
IFI16	1.00 ± 0.10	1.04 ± 0.09	1.00 ± 0.17	0.93 ± 0.18	1.00 ± 0.06	0.96 ± 0.05
IFI35	1.00 ± 0.14	0.99 ± 0.11	1.00 ± 0.14	0.80 ± 0.18#	1.00 ± 0.07	**0.78 ± 0.07***
IFI44	1.00 ± 0.48	0.54 ± 0.10	1.00 ± 0.15	**0.49 ± 0.08#****	1.00 ± 0.12	**0.60 ± 0.05****
IFI44L	1.00 ± 0.54	0.45 ± 0.09	1.00 ± 0.24#	0.58 ± 0.09#	1.00 ± 0.19	0.66 ± 0.09
IFIH1	1.00 ± 0.21	0.98 ± 0.09	1.00 ± 0.69	0.47 ± 0.30	1.00 ± 0.09	0.77 ± 0.07
IFIT2	1.00 ± 0.26	0.77 ± 0.09	1.00 ± 0.19#	0.75 ± 0.18#	1.00 ± 0.11	**0.78 ± 0.13***
IFIT3	1.00 ± 0.29	0.73 ± 0.11	1.00 ± 0.09	**0.55 ± 0.07*****	1.00 ± 0.06	**0.77 ± 0.08***
IFIT5	1.00 ± 0.26	0.79 ± 0.10	1.00 ± 0.16	0.64 ± 0.14#	1.00 ± 0.06	**0.72 ± 0.08****
IFITM3	1.00 ± 0.28	0.57 ± 0.10	1.00 ± 0.18	0.83 ± 0.14	1.00 ± 0.06	0.94 ± 0.07
INDO	1.00 ± 0.13	0.73 ± 0.10	n.d.#	n.d.#	1.00 ± 0.12#	0.88 ± 0.00#
IRF7	1.00 ± 0.16	0.80 ± 0.07	n.d.#	n.d.#	1.00 ± 0.15#	0.94 ± 0.17#
OAS1	1.00 ± 0.36	0.68 ± 0.09	n.d.#	n.d.#	1.00 ± 0.10	**0.67 ± 0.05****
OAS2	1.00 ± 0.37	0.68 ± 0.06	n.d.#	n.d.#	1.00 ± 0.16	0.70 ± 0.12#
OAS3	1.00 ± 0.48	0.50 ± 0.07	n.d.#	n.d.#	1.00 ± 0.14	0.77 ± 0.09
SOCS1	1.00 ± 0.07	0.87 ± 0.07	1.00 ± 0.22#	0.92 ± 0.24#	1.00 ± 0.08	0.91 ± 0.09
STAT1	1.00 ± 0.16	1.18 ± 0.10	1.00 ± 0.09	0.87 ± 0.16	1.00 ± 0.05	**0.87 ± 0.12***
STAT2	1.00 ± 0.16	1.07 ± 0.10	1.00 ± 0.14#	0.98 ± 0.12#	1.00 ± 0.05	0.97 ± 0.12
TAP1	1.00 ± 0.11	1.08 ± 0.10	1.00 ± 0.23#	0.95 ± 0.15#	1.00 ± 0.09	0.90 ± 0.10
TAP2	1.00 ± 0.12	1.08 ± 0.12	n.d.#	n.d.#	1.00 ± 0.08	0.92 ± 0.16
Apoptosis / Survival						
CASP8	1.00 ± 0.05	1.09 ± 0.07	n.d.#	n.d.#	1.00 ± 0.07	0.95 ± 0.06
BCL2	1.00 ± 0.12	1.19 ± 0.12	1.00 ± 0.21	0.47 ± 0.08	1.00 ± 0.06	1.08 ± 0.11
FASLG	1.00 ± 0.00#	1.13 ± 0.13#	n.d.#	n.d.#	n.d.#	n.d.#
FLCN1	1.00 ± 0.16#	1.03 ± 0.22#	n.d.#	n.d.#	1.00 ± 0.07	0.81 ± 0.09
TNFRSF1A	1.00 ± 0.07	0.99 ± 0.04	1.00 ± 0.14	**0.62 ± 0.12#***	1.00 ± 0.02	1.02 ± 0.06
TNFRSF1B	1.00 ± 0.06	0.93 ± 0.05	n.d.#	n.d.#	1.00 ± 0.09	0.86 ± 0.06
Small GTPases / (Rho)GTPase activating proteins						
ASAP1	1.00 ± 0.05	1.08 ± 0.05	1.00 ± 0.24	1.05 ± 0.31	1.00 ± 0.05	0.87 ± 0.09
RAB13	1.00 ± 0.19	1.11 ± 0.24	1.00 ± 0.14#	0.86 ± 0.14#	1.00 ± 0.05	0.90 ± 0.05
RAB24	1.00 ± 0.07	1.20 ± 0.24	1.00 ± 0.00#	1.21 ± 0.14#	1.00 ± 0.06	0.93 ± 0.08
RAB33A	1.00 ± 0.21	0.85 ± 0.05	1.00 ± 0.15#	0.92 ± 0.14#	1.00 ± 0.16#	2.20 ± 0.80#
TAGAP	1.00 ± 0.08	1.12 ± 0.07	n.d.#	n.d.#	1.00 ± 0.17	0.88 ± 0.13
TBC1D7	1.00 ± 0.17#	0.82 ± 0.13#	n.d.#	n.d.#	1.00 ± 0.15#	0.99 ± 0.15#

	Blood		Muscle		WAT	
Gene	White Caucasians	South Asians	White Caucasians	South Asians	White Caucasians	South Asians
Chemokines						
CCL11	n.d.#	n.d.#	n.d.#	n.d.#	1.00 ± 0.46#	2.51 ± 1.97#
CCL13	n.d.#	n.d.#	n.d.#	n.d.#	1.00 ± 0.37	0.96 ± 0.23
CCL19	1.00 ± 0.08	**0.53 ± 0.10***	1.00 ± 0.10#	1.17 ± 0.19#	1.00 ± 0.15	1.17 ± 0.18
CXCL9	1.00 ± 0.13#	0.84 ± 0.10#	n.d.#	n.d.#	1.00 ± 0.09	**0.72 ± 0.11***
CX3CL1	n.d.#	n.d.#	1.00 ± 0.22#	0.69 ± 0.00#	1.00 ± 0.14	0.86 ± 0.10
Cell growth / proliferation						
BMP6	1.00 ± 0.06	0.94 ± 0.06	1.00 ± 0.15	0.88 ± 0.25	1.00 ± 0.11	1.03 ± 0.09
TGFBR2	1.00 ± 0.05	**0.88 ± 0.09***	1.00 ± 0.13#	0.96 ± 0.16#	1.00 ± 0.06	**0.83 ± 0.04***
AREG	n.d.#	n.d.#	n.d.#	n.d.#	n.d.#	n.d.#
EGF	1.00 ± 0.18	0.85 ± 0.14	1.00 ± 0.16	1.23 ± 0.40#	1.00 ± 0.12	1.06 ± 0.16
VEGF	1.00 ± 0.22#	0.50 ± 0.00#	1.00 ± 0.27	1.09 ± 0.33	1.00 ± 0.15	0.70 ± 0.06
Cell activation						
HCK	1.00 ± 0.04	0.92 ± 0.03	n.d.#	n.d.#	1.00 ± 0.11	0.92 ± 0.11
LYN	1.00 ± 0.06	1.02 ± 0.04	n.d.#	n.d.#	1.00 ± 0.08	0.89 ± 0.10
SLAMF7	1.00 ± 0.16	1.23 ± 0.10	n.d.#	n.d.#	1.00 ± 0.11	1.02 ± 0.15
Transcriptional regulators / activators						
CAMTA1	n.d.#	n.d.#	n.d.#	n.d.#	n.d.#	n.d.#
TWIST1	n.d.#	n.d.#	n.d.#	n.d.#	1.00 ± 0.16#	1.05 ± 0.12
ZNF331	1.00 ± 0.25#	0.84 ± 0.12	n.d.#	n.d.#	1.00 ± 0.07	0.88 ± 0.06
ZNF532	1.00 ± 0.18	1.06 ± 0.28#	n.d.#	n.d.#	1.00 ± 0.05	0.92 ± 0.05
Intracellular transport						
SEC14L1	1.00 ± 0.05	0.95 ± 0.08	1.00 ± 0.12	**0.67 ± 0.07***	1.00 ± 0.10	0.86 ± 0.04
KIF1B	1.00 ± 0.04	1.05 ± 0.06	1.00 ± 0.12	0.94 ± 0.20	1.00 ± 0.04	0.93 ± 0.05
Inflammation						
DSE	1.00 ± 0.09	**0.76 ± 0.06***	1.00 ± 0.21	0.67 ± 0.16#	1.00 ± 0.09	1.07 ± 0.07
MMP9	1.00 ± 0.22	0.67 ± 0.10	n.d.#	n.d.#	1.00 ± 0.19	1.33 ± 0.43
SPP1	n.d.#	n.d.#	n.d.#	n.d.#	1.00 ± 0.30	0.82 ± 0.30
TIMP2	1.00 ± 0.06	0.97 ± 0.04	1.00 ± 0.13	0.68 ± 0.07	1.00 ± 0.07	1.02 ± 0.06
TNIP1	1.00 ± 0.07	1.01 ± 0.05	1.00 ± 0.31	**1.49 ± 0.41***	1.00 ± 0.08	0.92 ± 0.07
FPR1	1.00 ± 0.05	**0.71 ± 0.06****	1.00 ± 0.24#	0.47 ± 0.00#	1.00 ± 0.08	0.83 ± 0.09
BPI	1.00 ± 0.13	0.88 ± 0.05	1.00 ± 0.21#	0.55 ± 0.08#	1.00 ± 0.14	**0.54 ± 0.06****
Mitochondrial Stress / Proteasome						
HPRT	1.00 ± 0.12	1.25 ± 0.09	n.d.#	n.d.#	1.00 ± 0.12	1.13 ± 0.17