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Macrophage gene expression in adipose tissue is associated with insulin sensitivity and serum lipid levels independent of obesity

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Abstract

Objective—Obesity is linked to both increased metabolic disturbances and increased adipose tissue macrophage infiltration. However, whether macrophage infiltration directly influences human metabolism is unclear. The aim of this study was to investigate if there are obesity-independent links between adipose tissue macrophages and metabolic disturbances.

Design and Methods—Expression of macrophage markers in adipose tissue was analyzed by DNA microarrays in the SOS Sib Pair study and in patients with type 2 diabetes and a BMI-matched healthy control group.

Results—The expression of macrophage markers in adipose tissue was increased in obesity and associated with several metabolic and anthropometric measurements. After adjustment for BMI, the expression remained associated with insulin sensitivity, serum levels of insulin, C-peptide, high density lipoprotein cholesterol (HDL-cholesterol) and triglycerides. In addition, the expression of most macrophage markers was significantly increased in patients with type 2 diabetes compared to the control group.

Conclusion—Our study shows that infiltration of macrophages in human adipose tissue, estimated by the expression of macrophage markers, is increased in subjects with obesity and diabetes and associated with insulin sensitivity and serum lipid levels independent of BMI. This

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indicates that adipose tissue macrophages may contribute to the development of insulin resistance and dyslipidemia.

Introduction

The prevalence of obesity has increased during the last 20-30 years (1, 2). Today, approximately 1.1 billion of the world's population are overweight (body mass index, BMI>25kg/m²) and 312 million are obese (BMI>30 kg/m²) (2, 3). Obesity is associated with dyslipidemia, hypertension and hyperglycemia, which are major risk factors for cardiovascular morbidity and mortality (3-6).

Obesity is also linked to low-grade inflammation, with elevated levels of inflammatory markers in the blood (7, 8) and increased infiltration of macrophages in the adipose tissue (9, 10). Accumulating evidence suggests that the adipose tissue inflammation causes metabolic complications (9, 10). However, it is still unclear what initiates the inflammation in adipose tissue and hypotheses for this include hypertrophy of the adipocytes, hypoxia, endoplasmic reticulum (ER) stress and altered secretion of chemotactic factors. (11-15).

Macrophages are present in the adipose tissue of lean individuals but are more interspersed compared to those found in obese individuals, where macrophages are commonly clustered around dying adipocytes (16). In mice, diet-induced obesity causes a phenotype switch in adipose tissue macrophages from an anti-inflammatory (M2) to a proinflammatory phenotype (M1), and this change could contribute to adipose tissue inflammation (17). Proinflammatory macrophages (M1) have also been shown to be increased in human obesity (18). However, in other human studies the macrophages that accumulate during the development of obesity have been described as M2 macrophages with a remodeling phenotype (19) or M2-like macrophages capable of producing inflammatory cytokines (20). Weight loss through bariatric surgery reduces the macrophage content in adipose tissue (18, 21) in parallel with the improvement of obesity-associated metabolic disturbances (22). However, whether macrophage infiltration directly influences metabolic disturbances is unclear. The aim of this study was therefore to investigate if there are obesity-independent links between macrophage gene expression in adipose tissue and metabolic disturbances.

Methods and procedures

The regional Ethics Committee in Gothenburg, Sweden approved the studies. Subjects received written and oral information before giving informed consent.

The SOS Sib Pair study

The Swedish Obese Subjects (SOS) Sib Pair study consists of 732 subjects in 154 families with BMI discordant sibling pairs (over 10 kg/m² difference in BMI) recruited from all over Sweden. Adipose tissue gene expression profiles were available from 357 individuals in the offspring group (Supplementary table S2 online) and from 88 individuals in the parent group (Supplementary table S3 online). For comparisons between lean and obese siblings, sex-discordant pairs were excluded and only the most BMI-discordant siblings in each family were used, which resulted in 78 pairs of sisters and 12 pairs of brothers (23). Subcutaneous adipose tissue biopsies from the abdomen were obtained by needle aspiration during local anesthesia, snap frozen in liquid nitrogen, stored at -80°C and subsequently used for mRNA extraction. Gene expression analysis was performed using U133Plus2.0 arrays (Affymetrix Santa Clara, CA) as previously described (24). All expression profiles were analyzed using the Robust Multichip Analysis (RMA) method in the Affymetrix Expression Console software (Affymetrix). RMA is an analysis procedure that normalizes and summarizes the microarray raw data to obtain signal values that are comparable between microarrays (25).

Blood samples were taken after an overnight fast and blood chemistry analyses were performed at the Department of Clinical Chemistry, Sahlgrenska University Hospital, accredited according to the international standard ISO/IEC 17025. Frequent-sampling intravenous glucose tolerance test (FSIVGTT) (26) was performed for the assessment of insulin sensitivity (S_i). In the morning, after an overnight fast, subjects were placed in a recumbent position, and intravenous catheters were placed in the antecubital vein of each arm. An insulin-modified FSIVGTT (27) was performed. Briefly, a bolus of glucose (300 mg/kg) was infused intravenously over 2 minutes. Twenty minutes after initiation of the glucose bolus, an intravenous dose of insulin was given (0.03 U/kg for subjects with BMI below 35 kg/m², and 0.05 U/kg for subjects with BMI greater or equal to 35 kg/m²). Blood samples were collected for plasma glucose and serum insulin determinations at -15, -10, -5, -1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 20, 22, 24, 25, 27, 30, 40, 50, 60, 70, 90, 100, 120, 140, 160, and 180 minutes, relative to the initiation of the glucose bolus (0 minutes). Data were subjected to minimal model analysis using the computer program MINMOD, (Millennium 6.02 version Los Angeles, California) to generate estimates of insulin sensitivity (S_i) (28). Body composition was analyzed with Dual-Energy X-ray Absorptiometry (DEXA) at the Sahlgrenska University Hospital (LUNAR DPX-L, Scanexport Medical, Helsingborg, Sweden).

Patients with type 2 diabetes

Six patients with recently diagnosed type 2 diabetes and seven BMI- and sex matched non-diabetic controls were recruited from Primary Health Care centers (Supplementary table S4 online). All patients were diagnosed according to the WHO-criteria (29). Blood samples were taken after an overnight fast and blood chemistry analyses were performed as above. Subcutaneous adipose tissue biopsies from the abdomen were obtained by needle aspiration and were immediately frozen in liquid nitrogen until RNA-extraction. Gene expression analysis was performed using GeneST1.0 arrays (Affymetrix) at the Uppsala Array Platform. All expression profiles were normalized using the RMA method (Affymetrix).

Selection and evaluation of macrophage genes

Thirty-one genes, previously used as macrophage markers (9, 10, 18, 30-33) were selected for evaluation. These putative macrophage markers were initially examined in a human immune cell transcriptome dataset (<http://www.ncbi.nlm.nih.gov/geo/>, GSE3982) to ensure that no other immune cell displayed high expression of these genes. Genes with at least 2-fold higher expression levels in macrophage-like cells (macrophages, LPS-stimulated macrophages, immature dendritic cells or LPS-stimulated dendritic cells) compared to other immune cells were selected for further evaluation.

Next, gene expression of putative macrophage markers in adipose tissue was evaluated in the offspring group of the SOS Sib Pair Study using a pair-wise association analysis between all the markers. The rationale for this analysis step was to exclude macrophage markers displaying low association to the other macrophage markers due to expression in adipocytes or other non-macrophage cell types in adipose tissue.

Statistical analysis

To obtain approximate normal distributions, data were transformed using Box-Cox power transformations. Subsequently, data were standardized to mean = 0 and variance = 1. A mixed model analysis was used to assess the association between adipose tissue gene expression of macrophage markers and anthropometric and metabolic measurements in the offspring group. Models were adjusted for age, sex, BMI and non-independence among related individuals. A general linear model was used for analysis in the parent group where no adjustment for non-independence among related individuals was needed. In both the

mixed model and general linear model analyses, the parameter estimate and the p-values were used to describe the relationship between macrophage markers and anthropometric and metabolic measurements. A significant p-value and a high parameter estimate indicate a strong association between the parameters. Ten individuals from the five largest families in the SOS Sib Pair offspring group were excluded in the mixed model analysis due to convergence problems. Differences in the expression of macrophage genes between lean and obese siblings in the SOS Sib Pair Study were analyzed with paired student's T-tests. Differences in the expression of macrophage genes between type 2 diabetes patients and their BMI-matched controls were analyzed with the Mann-Whitney U-test due to the small study population. P-values below 0.05 were considered significant. All statistical analyses were performed with PASW18 (IBM, Chicago, IL) and Statistical Analysis Software, SAS version 9.1 (SAS Institute Inc., Cary, NC).

Results

Selection of macrophage markers in human adipose tissue

A two-step procedure was used to evaluate 31 putative macrophage markers (supplementary table S1 online). In the first step, 19 of the markers were selected for further analysis as they displayed at least a 2-fold higher expression in macrophage-like cell types compared to other immune cells. These 19 putative markers were then evaluated using pair-wise association analysis of adipose tissue gene expression levels (Supplementary figure S1 online). One macrophage marker was removed due to low level of association to the other macrophage markers, resulting in a total of 18 macrophage markers that were used in the analysis (table 1).

Expression of macrophage markers in relation to metabolic and anthropometric measurements

Initially, the expression of the 18 selected macrophage markers in adipose tissue was analyzed in BMI discordant, sex matched sibling pairs from the SOS Sib Pair study. All of the 18 markers were significantly higher expressed in the obese compared to the lean siblings, and on average there was a 2-fold increase (Table 1).

The expression of the macrophage markers was significantly associated with all investigated metabolic and anthropometric measurements in the SOS Sib Pair offspring group (Fig. 1A). The strongest positive associations were observed for estimates of adiposity (BMI, fat mass and percent body fat) and for serum levels of insulin and C-peptide. Strong negative associations were observed between the expression of macrophage markers and HDL-cholesterol and insulin sensitivity assessed by minimal model. To determine the influence of obesity on the associations between macrophage markers and metabolic phenotypes, the same analysis was repeated with a BMI-adjustment. Overall, the associations were weakened by the BMI adjustment, however, the expression of macrophage markers remained significantly associated to insulin sensitivity, serum levels of insulin, C-peptide, HDL-cholesterol and triglycerides (Fig. 1B).

To validate the obesity-independent associations, the BMI-adjusted analysis was repeated in the parent group of the SOS Sib Pair study. In line with the results found in the SOS Sib Pair offspring group, the majority of the macrophage markers displayed significant obesity-independent associations with insulin sensitivity, serum levels of insulin, HDL-cholesterol and triglycerides (Fig. 2). For C-peptide and insulin sensitivity, eight out of the 18 markers displayed significant associations (Fig. 2).

Expression of macrophage markers in patients with type 2 diabetes

To further investigate the link between macrophages in adipose tissue and insulin sensitivity, we examined the expression of the macrophage markers in patients with type 2 diabetes and their BMI-matched non-diabetic control group. 15 of the 18 selected macrophage markers were significantly higher expressed in adipose tissue from the patients with type 2 diabetes compared to controls (Fig. 3).

Discussion

In this study, we show that expression of macrophage markers in adipose tissue is associated with metabolic disturbances independent of obesity. In both the offspring group and parental group of the Sib Pair study, we found significant associations between macrophage markers and insulin sensitivity, serum levels of insulin, HDL-cholesterol and triglycerides after adjustment for BMI. Hence, our results indicate that macrophages may play an active role in the development of metabolic disturbances.

Obesity is associated with several metabolic co-morbidities such as insulin resistance and dyslipidemia. However, as much as 30 % of the obese population is metabolically healthy, indicating that other factors apart from obesity itself are important for the development of metabolic disease (34). The increased macrophage infiltration in adipose tissue has been suggested as a cause of metabolic disturbances in human obesity (9, 10). Our results clearly support the link between macrophage infiltration and obesity as we find strong associations between macrophage markers and anthropometric and metabolic measurements. When adjusting our analysis for BMI, associations between macrophage markers and anthropometric measurements were lost. This is expected since BMI is tightly linked to other anthropometric measurements. However, associations with some metabolic measures (insulin, C-peptide, HDL, TG and Si) remained, suggesting that macrophages in adipose tissue play an active role in the development of metabolic disturbances. This is also supported by our results showing that the expression of macrophage markers was increased in patients with type 2 diabetes compared to a BMI-matched control group. Hence, increased adipose tissue macrophage infiltration may explain why some obese individuals develop metabolic disease whereas others remain metabolically healthy. It is unknown if macrophage infiltration precedes metabolic disturbances, but if it does, it is possible that estimation of macrophage content in adipose tissue could be used to predict the risk for metabolic disease in patients. In addition, if an active role of adipose tissue macrophages in development of metabolic disturbances is confirmed, this could be a future target for prevention of metabolic disease.

Studies in rodents have provided mechanistic insights into how adipose tissue macrophages may affect metabolic disturbances (12, 35). For example, over-expression of the monocyte chemoattractant protein 1 (MCP-1) in adipose tissue in mouse contributed to increased macrophage infiltration and increased insulin resistance. However, equivalent mechanistic studies on the role of adipose tissue macrophages in human metabolic disease are hard to perform. Previously, several studies have investigated the association between macrophage content in adipose tissue and insulin resistance (32, 36-38) as well as serum triglyceride and HDL cholesterol levels (33, 36). These studies indicate that macrophages may play a role in the development of human metabolic disturbances. However, they have been performed in relatively small populations and with some exceptions (33) the degree of obesity has not been taken into account in the analysis. Hence, our study represents the first systematic analysis of BMI-independent metabolic effects of adipose tissue macrophages in large, well-characterized study populations.

Macrophages show a high degree of plasticity and results from in vitro studies have suggested that distinct macrophage subsets, such as M1 and M2 macrophages, exist. However, in vivo, macrophages are most likely heterogeneous, and M1 and M2 macrophages probably represent the extreme ends of this polarization spectrum. Markers suggested to be specific for either M1 macrophages (e.g. CD40 and ITGAX) or M2 macrophages (e.g. MRC1, MSR1 and CD163) were included in the initial evaluation of markers. However, in the further analysis, markers representing M1 macrophages had to be excluded due to high expression in other immune cells. Unfortunately this limits the possibility to perform macrophage subset specific analysis of our data. Furthermore, it indicates that some, well-established markers for immunohistochemical or flow cytometric analyses of macrophage phenotypes are not optimal for gene expression analysis.

The strength of this study is that multiple macrophage markers were analyzed in large, well characterized groups of subjects. The limitations of the study are that we have used the expression of macrophage markers as a proxy for macrophage content in adipose tissue and that we have not analyzed the expression of the macrophage markers at the protein level. However, previous studies show that the expression of macrophage markers correlates well with actual macrophage infiltration in adipose tissue as analyzed by immunohistochemistry (30, 32, 33, 39).

In conclusion, our study shows that the macrophage content in adipose tissue, estimated by the expression of macrophage markers, is associated with insulin sensitivity and serum lipid levels independent of obesity. Hence, it is not only the adipose tissue quantity, but also the adipose tissue cellular composition, that may be of importance from a metabolic perspective.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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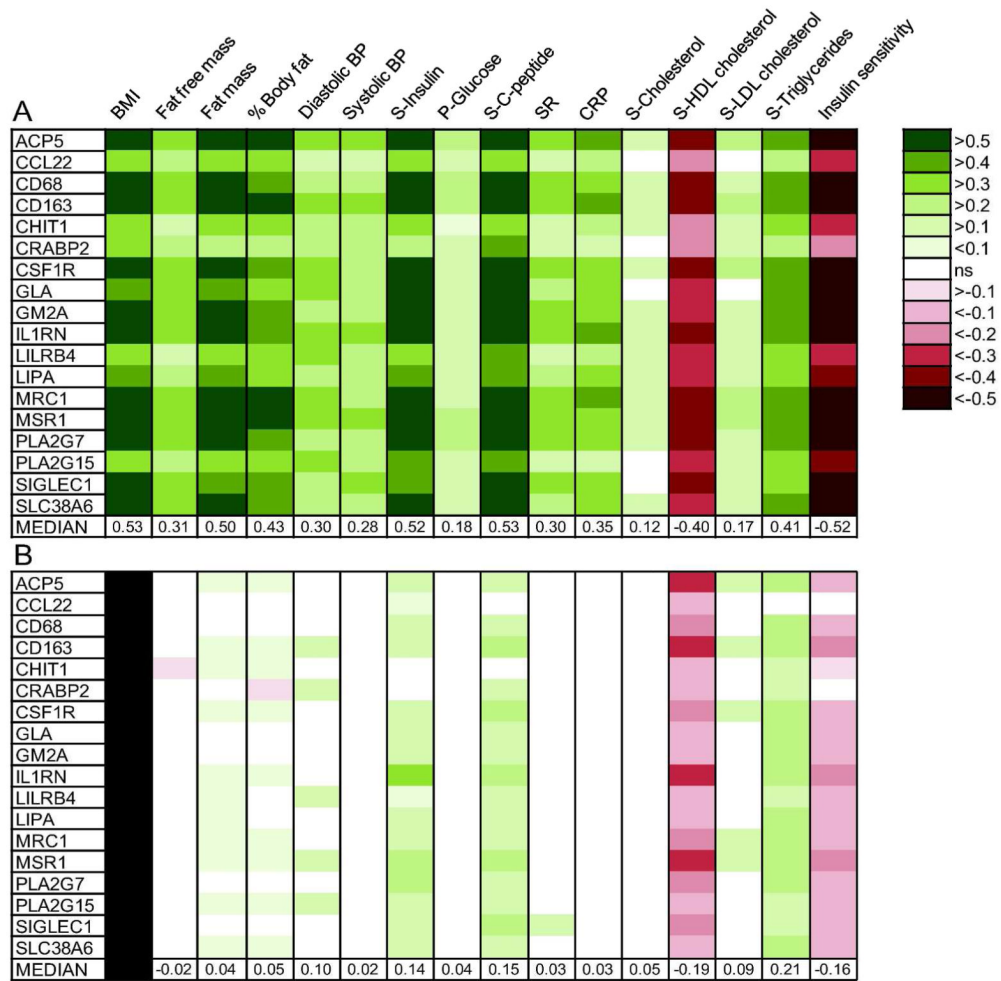


Figure 1.

Associations between adipose tissue expression of macrophage markers and metabolic and anthropometric measurements in the offspring group of the SOS Sib Pair study. Macrophage markers are indicated by their gene symbol. The colors represent the level of association (parameter estimate) to the various measurements. Green color represents a significant positive association and red color represents a significant negative association. White color represents non-significant association. A. Associations between expression of macrophage markers and anthropometric and metabolic measurements adjusted for age, sex and non independence among related individuals. B. Associations between expression of macrophage markers and anthropometric and metabolic measurements adjusted for BMI, age and sex and non independence among related individuals. The median parameter estimate is shown in the bottom row. BP = blood pressure, SR = sedimentation rate, CRP = C-reactive protein

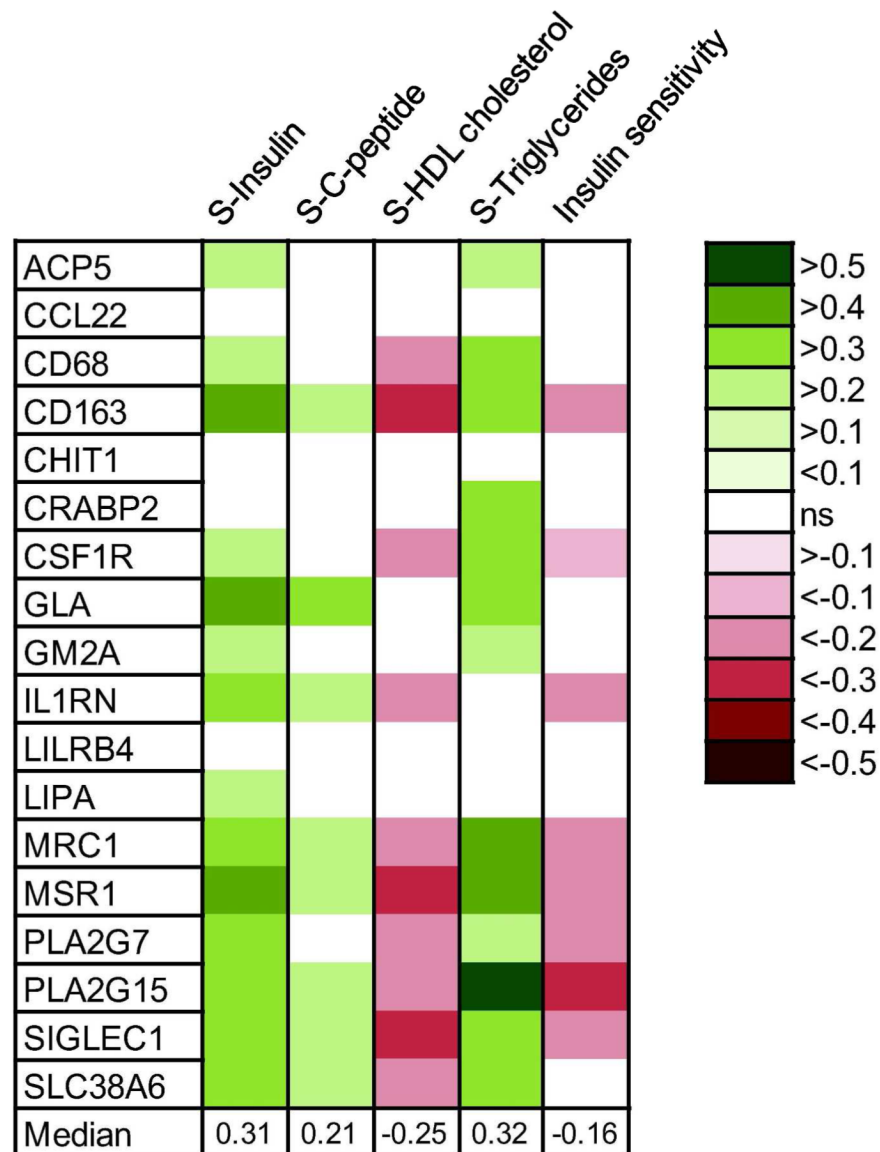


Figure 2.

Associations between adipose tissue expression of macrophage markers and metabolic measurements adjusted for BMI, age and sex in the parent group of the SOS Sib Pair study. Macrophage markers are displayed by their gene symbol. The colors represent the level of association (parameter estimate) to the various measurements. Green color represents a significant positive association and red color represents a significant negative association. White color represents non-significant association. The median parameter estimate is shown in the bottom row.

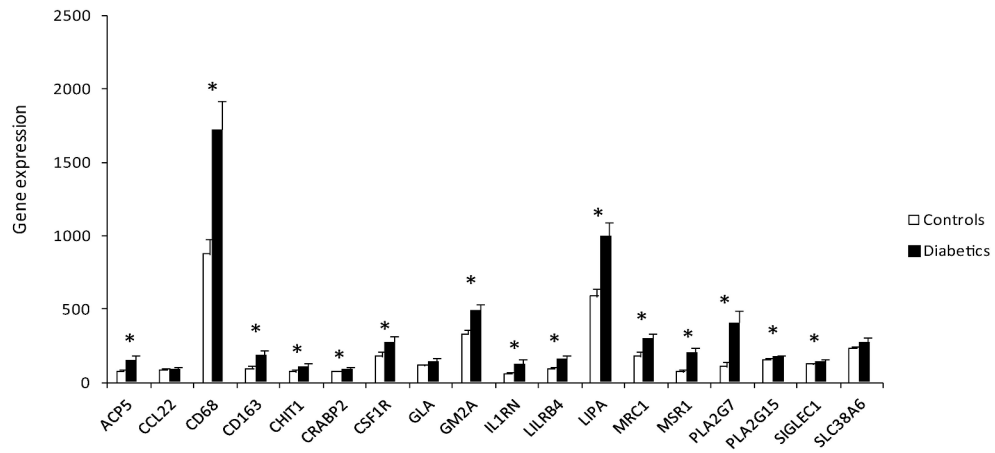


Figure 3. The expression of the 18 macrophage markers in adipose tissue of male type 2 diabetes patients (n=6) and a BMI- and sex matched control group (n= 7). *=p<0.05. Data are presented as mean expression ± SEM.

Table 1

The difference in expression of macrophage markers between lean and obese sibling pairs

	P-value	Fold change (FC)
ACP5	3.56×10^{-17}	3.37
CCL22	1.29×10^{-6}	1.33
CD68	1.02×10^{-13}	1.57
CD163	2.67×10^{-23}	2.64
CHIT1	5.91×10^{-8}	2.40
CRABP	21.19×10^{-5}	1.21
CSF1R	5.58×10^{-18}	1.70
GLA	2.01×10^{-9}	1.31
GM2A	1.85×10^{-15}	1.65
IL1RN	2.36×10^{-17}	4.63
LILRB4	3.79×10^{-6}	1.18
LIPA	2.45×10^{-9}	1.63
MRC1	3.70×10^{-19}	2.02
MSR1	3.47×10^{-20}	2.80
PLA2G7	1.85×10^{-11}	3.05
PLA2G15	9.16×10^{-7}	1.19
SIGLEC1	4.03×10^{-15}	1.47
SLC38A6	3.51×10^{-14}	1.50
Average FC		2.04 ± 0.22