ESM methods

Isolation and culture of adipose precursor cells

Adipose precursor cells were isolated from the subcutaneous biopsies. The fresh adipose tissue biopsy was cut into small pieces with sterile scissors and transferred to a sterile digestion solution (HAM-DMEM/F12 medium (Life technologies, CA, USA) containing 1 mg/ml collagenase II, and 15 mg/ml fatty acid-free bovine serum albumin (BSA, Sigma-Aldrich, MO, USA) and shaken for 20 minutes. The cell suspension (containing the stromal-vascular cells) was filtered through a cell strainer and the infranatant layer was transferred to a culture flask for ADSC propagation. Cells positive for the surface markers CD90 and CD166, and negative for CD45 and CD31 are considered a population of cells with a high adipogenic potential. We previously performed flow cytometry of the cells included in this study, and observed no difference in the percentage of cells with high adipogenic potential in cultures established from the stromal-vascular fraction from LBW and NBW donors (22). Studies were performed on passage 1 cells from all donors (i.e. cells that have only been frozen in liquid nitrogen once) to ensure comparability. The isolated ADSCs were cultured in proliferation media (DMEM/F12 media supplemented with 10% foetal bovine serum (FBS) (vol./vol,), 1% penicillin streptomycin (PS) (vol./vol,), and 1nmol/l fibroblast growth factor (FGF)) until they reached 100% confluence. Two days after confluence (designated day 0), ADSCs were cultured for three days in differentiation media (DMEM/F12 supplemented with 1% PS (vol./vol,), rosiglitazone (200 nmol/l), dexamethasone (0.1 μmol/l), insulin (100 nmol/L), Triiodothyronine (T₃) (2nmol/l), transferrin (10 μg/ml) and 3-isobuthyl-1-methylxanthine (IBMX) (540 μmol/l)). Hereafter, adipocytes were cultured for nine days in differentiation media without IBMX. Media was changed every 72 h and adipocytes were grown until day 12 when all cultures displayed adipocyte morphology with massive lipid droplet accumulation. ADSCs were harvested at 80% confluence (while still in proliferation media) and at day 12 of the differentiation protocol.
**Gene expression analysis**

Total RNA was extracted from ADSCs and mature adipocytes using TRIzol® (Invitrogen) according to the manufacturer’s instructions. RNA quality was assessed using a Bioanalyzer and all samples had an acceptable RIN score >9. In total, 47 samples (LBW: 11 ADSC samples and 12 mature adipocytes samples; NBW: 11 ADSC samples and 13 mature adipocytes samples) had sufficient amount of RNA (250 ng) to be included for gene expression analysis using the HumanHT-12 v4 Expression BeadChip (Illumina). LBW and NBW individuals were randomized throughout the chips. Preprocessing of gene expression data was performed using the lumi package in Bioconductor [1]. Individual probes were filtered based on mean detection p-values and all probes with a mean detection p-value ≤0.01 in at least 60% of the samples were considered detected. The data was background corrected, variance stabilized and quantile normalized using the lumiExpresso function. ComBat [2] was used to correct for batch effects. After preprocessing, gene expression data for 17,305 probes was obtained. Annotation of probes was based on the Bioconductor package lumiHumanAll.db [3]. DAVID Bioinformatics Resource 6.7 (http://david.abcc.ncifcrf.gov/) was used to identify functionally related gene groups among our significant reads (q<0.05). Genetic interaction networks between genes showing differential gene expression (q<0.05) were constructed using a GeneMANIA plugin version 2.3.3 (http://apps.cytoscape.org/apps/genemania) in Cytoscape [4, 5]. The initial GeneMANIA networks of genetic interactions were extended with regulatory interaction networks (RegIns) using the CyTargetLinker app (http://projects.bigcat.unimaas.nl/cytargetlinker/regins/) in Cytoscape.
DNA methylation arrays

DNA was isolated using Qiagen DNeasy® Blood & Tissue kit (cat 69506) and 500 ng were bisulfite treated using the EZ DNA methylation kit (Zymo Research, Orange, CA, USA). Genome-wide DNA methylation profiling in the ADSCs and mature adipocytes was assessed in all samples using the Infinium HumanMethylation450 BeadChip (Illumina) [6]. Samples were randomly placed on the chips while assuring even distribution of LBW and NBW individuals. Paired samples (ADSC and adipocytes from the same individual) were located adjacent on the same chip. The BeadChips were imaged with Illumina iScan. Raw fluorescence intensities of the scanned images were extracted with GenomeStudio Methylation module software, and the raw methylation score for each probe represented as methylation β-value was calculated (β-value=intensity of the Methylated allele (M)/intensity of the Unmethylated allele (U)+intensity of the Methylated allele (M)+100). All samples had high bisulfite conversion efficiency (intensity signal >4000) [7] and passed quality control steps in GenomeStudio where built in control probes for staining, hybridization, extension and specificity were examined.

DNA methylation data was exported from GenomeStudio and subsequent analyses were performed using Bioconductor [8] and the lumi package [1]. Methylation β-values were converted to M-values (M=\log_2(\beta/(1-\beta))). Before further preprocessing steps of the methylation data, we removed probes reported to be cross-reactive (≥49 bases) [9], probes directly affected by polymorphisms in the CpG site or the single base extension of Infinium Type I probes (European allele frequency >0.01) [9], probes mapped to incorrect genomic location [9], and the 65 random SNP probes included on the array. Individual probes were then filtered based on their mean detection P-value and all probes with a P-value<0.01 were considered detected and used for further analysis. The methylation data was background corrected by subtracting the median M-value of the 600 built in negative controls and was further normalized using quantile normalization [10] and
beta-mixture quantile (BMIQ) normalization [11]. ComBat [2] was used to correct for batch effects. After preprocessing, DNA methylation data of 446,548 CpG sites and 1,017 non-CpG sites were obtained from adipocytes before and after differentiation of 13 LBW and 13 NBW subjects. Since β-values are more biological interpretable, M-values were reconverted back to β-values for presentation of results and figures \( (\beta = 2^M/(2^M+1)) \)[12]. Annotation of probes was based on the HumanMethylation450 v1.2 Manifest File (Illumina).

Reference List


ESM Tables

ESM Table 1. Primer sequences

<table>
<thead>
<tr>
<th>Primers for RT-PCR</th>
<th>Primer Sequence (RNA), 5′→3′</th>
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<td>CCNT2</td>
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ESM Table 2: Expression in NBW vs LBW ADSCs. See Excel file

ESM Table 3: Methylation in NBW vs LBW ADSCs. See Excel file

ESM Table 4: See Excel file. Common gene variants in the CCNT2 region associated with cardiometabolic traits. The CCNT2 region was defined as 2q21.3 using NCBI’s Variation Viewer. This yielded 7,446 unique SNPs of which 86 common variants had been studied in previous GWAS meta-analyses for coronary artery disease, type 2 diabetes, fasting glucose, fasting insulin, HOMA-IR, 2-hour glucose, BMI and WHR. The original publically available data from the CARDIoGRAM, DIAGRAM, MAGIC and GIANT consortia are shown. NA indicates no overlap between CCNT2 region variant and GWAS.

ESM Table 5: See Excel file. Common gene variants in the STAT2 region associated with cardiometabolic traits. The STAT2 region was defined as 12q13.3 using NCBI’s Variation Viewer. This yielded 6,892 unique SNPs of which 29 common variants had been studied in previous GWAS meta-analyses for coronary artery disease, type 2 diabetes, fasting glucose, fasting insulin, HOMA-IR, 2-hour glucose, BMI and WHR. The original publically available data from the CARDIoGRAM, DIAGRAM, MAGIC and GIANT consortia are shown. NA indicates no overlap between STAT2 region variant and GWAS.

ESM Table 6: Expression in NBW vs LBW adipocytes. See Excel file

ESM Table 7: Methylation in NBW vs LBW adipocytes. See Excel file