A Genome-Wide mQTL Analysis in Human Adipose Tissue Identifies Genetic Variants Associated with DNA Methylation, Gene Expression and Metabolic Traits

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Abstract

Little is known about the extent to which interactions between genetics and epigenetics may affect the risk of complex metabolic diseases and/or their intermediary phenotypes. We performed a genome-wide DNA methylation quantitative trait locus (mQTL) analysis in human adipose tissue of 119 men, where 592,794 single nucleotide polymorphisms (SNPs) were related to DNA methylation of 477,891 CpG sites, covering 99% of RefSeq genes. SNPs in significant mQTLs were further related to gene expression in adipose tissue and obesity related traits. We found 101,911 SNP-CpG pairs (mQTLs) in cis and 5,342 SNP-CpG pairs in trans showing significant associations between genotype and DNA methylation in adipose tissue after correction for multiple testing, where cis is defined as distance less than 500 kb between a SNP and CpG site. These mQTLs were further related to gene expression in adipose tissue and obesity related traits. We found 101,911 SNP-CpG pairs (mQTLs) in cis and 5,342 SNP-CpG pairs in trans showing significant associations between genotype and DNA methylation in adipose tissue after correction for multiple testing, where cis is defined as distance less than 500 kb between a SNP and CpG site. These mQTLs include reported obesity, lipid and type 2 diabetes loci, e.g. ADCY3/POMC, APOA5, CETP, FADS2, GCKR, SORT1 and LEPR. Significant mQTLs were overrepresented in intergenic regions meanwhile underrepresented in promoter regions and CpG islands. We further identified 635 SNPs in significant cis-mQTLs associated with expression of 86 genes in adipose tissue including CHRNA5, G6PC2, GPX7, RPL27A, THNSL2 and ZFP57. SNPs in significant mQTLs were also associated with body mass index (BMI), lipid traits and glucose and insulin levels in our study cohort and public available consortia data. Importantly, the Causal Inference Test (CIT) demonstrates how genetic variants mediate their effects on metabolic traits (e.g. BMI, cholesterol, high-density lipoprotein (HDL), hemoglobin A1c (HbA1c) and homeostatic model assessment of insulin resistance (HOMA-IR)) via altered DNA methylation in human adipose tissue. This study identifies genome-wide interactions between genetic and epigenetic variation in both...
Introduction

Genetic factors contribute to the risk of complex metabolic diseases such as obesity and type 2 diabetes. Although genome-wide association studies (GWAS) have identified numerous genetic loci influencing the risk of developing obesity and type 2 diabetes, only a few of these loci have been linked to the molecular mechanisms contributing to the phenotype outcome [1]. Moreover, the identified genetic loci do only explain a modest proportion of the estimated heritability of these diseases and additional genetic mechanisms remain to be found. These may include genetic variants interacting with epigenetic modifications.

The phenomenon of epigenetic modifications are of interest to study for their possible involvement in phenotype transmission and predisposition to complex human diseases, including obesity and type 2 diabetes [2,3]. Epigenetics has been defined as heritable changes in gene function that occur without alterations in the DNA sequence and includes the molecular mechanism of DNA methylation [4]. In differentiated mammalian cells, DNA methylation occurs primarily at cytosines in CG dinucleotides, so called CpG methylation, which is associated with regulation of cell specific gene expression [5,6]. DNA methylation patterns are mainly established early in life, but may also be dynamic and change in response to environmental stimulations such as diet and exercise [7–10]. Concurrently, once epigenetic modifications are introduced they can be stable and inherited [11,12], making epigenetics a potentially important pathogenic mechanism in complex metabolic diseases. Interestingly, twin studies provide evidence for an underlying genetic effect on DNA methylation patterns [13–16]. For example using monozygotic and dizygotic twins, Grundberg et al showed that as much as 37% of the methylation variance can be attributed to genetic factors, which is in line with previous studies [15,16]. In addition, recent studies showed that common genetic variation regulates DNA methylation levels, so called methylation quantitative trait loci (mQTLs) [16–20]. However, most of these studies have been limited to analyses of ~0.1% of human CpG sites in promoter regions [17–19] or restricted to SNPs located within 100 kb from analyzed CpG sites [16]. It remains to be tested if genetic and epigenetic variation interacts throughout the genome in human adipose tissue and subsequently affect gene expression and metabolic traits such as BMI, lipid levels and hemoglobin A1c (HbA1c) in the studied individuals.

The aim of the present study was therefore to perform a genome-wide mQTL analysis in human adipose tissue, investigating both cis and trans effects of genetic variation on DNA methylation covering most genes and regions in the human genome. Identified mQTLs were followed-up and related to gene expression in adipose tissue. Additionally, since the adipose tissue contributes to whole body energy homeostasis by glucose uptake, triglyceride storage and adipokine secretion, we investigated if the identified SNPs in significant mQTLs affect metabolic traits that are associated with increased risk of obesity and type 2 diabetes in the studied cohort. We further used a causal inference test (CIT) [21] to model the potential causal relationships between genotype, DNA methylation and metabolic phenotypes.

The present study provides the first detailed map of genetic loci in both cis and trans positions affecting the genome-wide DNA methylation pattern in human adipose tissue as well as numerous metabolic traits. Identified mQTLs cover known lipid, obesity and diabetes loci. Our study highlights that interaction analysis between genetic and epigenetic variation in a tissue of
relevance for metabolic diseases may give new insights to biological processes affecting disease susceptibility.

**Results**

**Associations between genetic variation and DNA methylation in human adipose tissue—a genome-wide mQTL analysis**

To examine and map underlying genetic control of DNA methylation patterns in human adipose tissue, we performed a genome-wide mQTL analysis (Fig 1). While most previous mQTL studies have been limited to analysis of ~0.1% of human CpG sites [17–19] or SNPs within 100 kb from analyzed CpG sites [16] we performed the first combined cis- and trans-mQTL analysis covering DNA methylation of most genes and genomic regions in human adipose tissue of 119 Scandinavian men (Table 1). Here, we pairwise associated genotype data of 592,794 common SNPs (MAF > 0.05) with DNA methylation of 477,891 CpG sites throughout the human genome using a linear regression model including sub-cohort, age and BMI as covariates.

The cis-mQTL analysis was limited to SNPs located within 500 kb of either side of the analyzed CpG sites. Here, we detected 101,911 SNP-CpG pairs (mQTLs) showing significant associations between genotype and the degree of DNA methylation after correction for multiple

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Fig 1. Analysis flowchart of the study.

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testing (see Methods), corresponding to 51,143 unique SNPs and 15,208 unique CpG sites (Table 2 and S1 Table). Of these 15,208 significant CpG sites, 10,064 were annotated to 5,589 unique genes (Table 2) and 5,144 CpG sites were annotated to intergenic regions. The most and least significant cis-mQTLs are shown in Fig 2A–2B.

Previously, we reported that approximately 50% of type 2 diabetes associated SNPs identified by GWAS either introduce or remove a CpG site, a so called CpG-SNP. These CpG-SNPs were further associated with differential DNA methylation of the CpG-SNP site in human pancreatic islets [22]. Among the significant cis-mQTLs in the present study, 447 SNPs were located within a CpG site, i.e. the distance between a SNP and CpG site is 0 or 1 and thereby remove or introduce a CpG site–CpG-SNPs (S1 Table). The most significant mQTL among these 447 cis-mQTLs is presented in Fig 2C.

When a distance analysis was performed, we found an overrepresentation (p < 2.2−16) of SNPs in significant cis-mQTL located close to the CpG site (Fig 2D–2E), with a median distance between SNPs and CpG sites of significant cis-mQTLs of 29.6 kb. Moreover, the strongest association signals were found for SNPs located close to a CpG site (Fig 2F).

In the trans-mQTL analysis, including SNPs located more than 500 kb from the analyzed CpG sites, we identified 5,342 SNP-CpG pairs showing significant associations between genotypes and the degree of DNA methylation in adipose tissue after correction for multiple testing.

Table 1. Characteristics of 119 Scandinavian men included in the mQTL analysis.

| Phenotype             | Mean ± SD | Min  | 1st quartile | Median | 3rd quartile | Max  |
|-----------------------|-----------|------|--------------|--------|--------------|------|
| Age (years)           | 31.03 ± 12.3 | 22   | 24           | 25     | 35           | 80   |
| Fasting Glucose (mmol/l) | 4.76 ± 0.64 | 3.2  | 4.4          | 4.7    | 5            | 7    |
| Fasting Insulin (pmol/l)  | 37.29 ± 22.43 | 8   | 23.1         | 33     | 43.3         | 181.3|
| Weight (kg)           | 80.86 ± 11.6 | 57.2 | 72.6         | 80.4   | 89.57        | 112.7|
| BMI (kg/m²)           | 24.91 ± 3.7 | 16.4 | 22.2         | 24.6   | 27.15        | 39   |
| Waist (cm)            | 90.31 ± 11.5 | 68   | 80.75        | 91     | 98.25        | 129  |
| Hip (cm)              | 97.55 ± 8.8  | 78   | 91.75        | 98     | 104.2        | 113  |
| Waist-Hip ratio       | 0.9 ± 0.06   | 0.79 | 0.87         | 0.9    | 0.92         | 1    |
| Cholesterol (mmol/l)  | 4.5 ± 0.84   | 2.1  | 3.9          | 4.5    | 5.1          | 7.1  |
| Triglycerides (mmol/l) | 1.14 ± 0.66  | 0.3  | 0.72         | 1      | 1.3          | 4.9  |
| HDL (mmol/l)          | 1.16 ± 0.2   | 0.5  | 1            | 1.13   | 1.37         | 1.86 |
| LDL (mmol/l)          | 2.8 ± 0.77   | 1    | 2.3          | 2.8    | 3.5          | 4.7  |
| HbA1c (%)             | 4.93 ± 0.48  | 3.7  | 4.7          | 5      | 5.2          | 6.4  |
| HOMA-IR               | 1.15 ± 0.78  | 0.2  | 0.7          | 1      | 1.4          | 6.5  |
| HOMA-B                | 133.69 ± 226.33 | 19.2 | 56           | 75.6   | 118.9        | 1834 |

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Table 2. Number of significant mQTL results in human adipose tissue.

|                  | cis-mQTL | trans-mQTL |
|------------------|----------|------------|
| SNP-CpG pairs    | 101,911  | 5,342      |
| SNPs             | 51,143   | 2,735      |
| CpG sites        | 15,208   | 596        |
| Unique genes     | 5,589    | 375        |

Significance threshold < 0.05 after Bonferroni correction for multiple testing.
Correction value cis = 104,023,091
Correction value trans = 211,781,637,483.

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Boxplots of (a) the top cis-mQTL, (b) the bottom cis-mQTL, and (c) the top cis-mQTL where the SNP introduces or removes a CpG site (CpG-SNP), showing significant associations between SNPs (genotype groups, x-axis) and DNA methylation of CpG sites (% y-axis). The frequency of associations (y-axis) is plotted in relation to the relative distance between SNPs and CpG sites (kb, x-axis) of significant cis-mQTLs. In (d) the full cis-mQTL distance of 500 kb is represented and the frequency of significant cis-mQTLs within each distance bin of 10kb are plotted, and, in (e) the region of 0-50kb is zoomed and the frequency of significant cis-mQTLs within each distance bin of 1kb is plotted. (f) Histogram showing the strength of association (-log_{10} p-value, y-axis) in relation to distance between SNP and CpG site (kb, x-axis) of significant cis-mQTLs. The most frequent and strongest association signals of cis-mQTLs are shown within SNPs located close to CpG sites. (g-h) Boxplots of (g) the top trans-mQTL, and (h) the bottom trans-mQTL, showing significant associations between SNPs (genotype groups, x-axis) and DNA methylation of CpG sites (% y-axis). pcorr, p-values have been corrected for multiple testing by a modified Bonferroni correction where the LD structure of SNPs is taken into account (see methods).

Genomic distribution of significant mQTLs in human adipose tissue

DNA methylation in proximal promoter and/or enhancer regions is generally thought to have silencing effects on gene transcription, meanwhile DNA methylation in the gene body might stimulate transcriptional elongation and contribute to alternative splicing events [6]. Giving the various functions of DNA methylation in the context of genomic regions, it is of interest to study the underlying mechanisms regulating DNA methylation patterns in different genomic regions. We therefore studied the chromosomal and genomic distribution of CpG sites in significant mQTLs in human adipose tissue. To determine whether the genomic distribution of CpG sites in significant mQTLs differ significantly from all analyzed CpG sites on the array, we performed chi-squared tests. The chromosomal distribution of CpG sites in significant cis- and trans-mQTLs is shown in Fig 3A. We found an overrepresentation of CpG sites in significant cis-mQTLs on chromosome 6, 7, 8, 13 and 21 together with an underrepresentation on chromosomes 1, 2, 3, 11, 12, 14, 15, 17, 18, 19, 20 and X when compared to the chromosomal distribution of all analyzed CpG sites (Fig 3A). The highest deviation from expectation of CpGs in significant cis-mQTLs was observed on chromosome 6 (p-value = 3.4x10^{-50}), where the highly polymorphic HLA region is located, a genomic region linked to numerous autoimmune diseases [23,24]. CpG sites in significant trans-mQTLs were overrepresented on chromosomes 6 and Y while underrepresented on chromosomes 9 and 14 (Fig 3A).

Furthermore, the Infinium HumanMethylation450 BeadChip estimates DNA methylation in several genomic features and the analyzed CpG sites have been annotated based on their genomic location in relation to the nearest gene including genomic regions TSS1500 and TSS200 (1500–201 and 200–0 bases upstream of transcription start site (TSS), respectively), 5'UTR (untranslated region), 1st exon, gene body, 3'UTR and intergenic regions [25]. In the present study, CpG sites in significant cis-mQTLs were overrepresented in the intergenic regions and gene body, while significantly underrepresented in the TSS1500, TSS200, 5'UTR, 1st exon and 3'UTR (Fig 3B). Among significant trans-mQTLs, we found an overrepresentation of CpGs in the intergenic region and underrepresentation in TSS1500 and gene body (Fig 3B).

The analyzed CpG sites have also been annotated based on their relation to CpG islands, including the following regions: CpG islands, northern and southern shores, northern and southern shelves and open sea [25]. For CpG sites in significant cis-mQTLs, we found an overrepresentation in the open sea, northern- and southern shores as well as in southern shelf...
Moreover, an underrepresentation was found in CpG islands (Fig 3C). CpGs in significant trans-mQTLs showed overrepresentation in the open sea and underrepresentation in northern shore as well as southern shelf (Fig 3C).

Next, we performed a KEGG (Kyoto Encyclopaedia of Genes and Genomes) pathway analysis to identify cellular components or biological pathways which show enrichment among
genes identified in *cis*- and *trans*- mQTL analyses in human adipose tissue. Using WebGestalt [26], we identified 172 significant (FDR < 0.05) KEGG pathways enriched among 5,589 genes annotated to significant *cis* mQTLs (S3 Table), including Metabolic pathways (P_{adj} = 6.3x10^{-15}) and Pathways in Cancer (P_{adj} = 7.3x10^{-42}) were found among the most enriched KEGG pathways (S3 Table). Moreover, 25 KEGG pathways were enriched among 375 genes annotated to significant *trans* mQTLs (S3 Table).

**Candidate loci for obesity and diabetes related traits are detected among mQTLs in human adipose tissue**

Numerous SNPs associated with obesity, type 2 diabetes and related traits have previously been identified by GWAS [1]. However, the molecular mechanisms explaining how most of these SNPs affect gene function and disease pathology remain scarce. We therefore tested if identified SNPs in significant mQTLs in adipose tissue overlap with loci previously reported to associate with obesity, type 2 diabetes or obesity/diabetes related traits in the GWAS catalog (p < 10^{-5}) [27]. Out of the SNPs significantly associated with DNA methylation in the *cis-*mQTL analysis and when taking proxy SNPs into account (R^2>0.8, see Methods), 19,706 overall, we found 231 SNPs of significant mQTLs that overlapped with at least one of the 2138 reported disease or trait locus identified in the GWAS catalog (S4 Table), which constitutes 1.17% of *cis*-mQTL SNPs and 10.8% of GWAS catalog SNPs. Representative mQTLs for some of these loci are shown in Fig 4A–4J. These mQTLs include POMC and LEPR, which encode proopiomelanocortin and the leptin receptor, respectively. Mutations in both these genes have been associated with early onset obesity [28]. We also present mQTLs covering GIPR (encoding gastric inhibitory polypeptide receptor), PARP4 (encoding poly(ADP-ribosyl)transferase-like 1 protein), CEPT (encoding cholesteryl ester transfer protein), APOA5 (encoding apolipoprotein A5), SORT1 (encoding sortilin 1), ACADS (encoding acyl-CoA dehydrogenase) and GRB10 (encoding growth factor receptor bound protein 10). SNPs in these loci have previously been associated with BMI, T2D and/or obesity- and lipid-related traits [29–36].

Of SNPs in significant *trans*-mQTLs, we found 4 SNPs overlapping with reported obesity loci in the GWAS catalog (Fig 4K and S5 Table).

**The impact of identified mQTLs on mRNA expression in human adipose tissue**

It is well established that mRNA expression is regulated by both genetic variation and DNA methylation independently [4,37]. However, the insights of how genetic and epigenetic variation interacts to influence gene expression remain limited. In order to study the impact of identified mQTLs on mRNA expression in human adipose tissue, we performed a follow-up eQTL analysis in 118 samples with available microarray expression data (out of original 119 samples). First, we related the 51,143 unique SNPs, showing significant association with DNA methylation in the *cis*-mQTL analysis, with mRNA expression of genes within 500kb (*cis*-distance). In the eQTL analysis of significant *cis*-mQTL SNPs, we identified 926 SNP-mRNA transcript pairs showing significant associations between genotypes and mRNA expression levels after correction for multiple testing (see Methods). These correspond to 635 unique SNPs and 86 unique genes, including CHRNA5, G6PC2, GPX7, RPL27A, THNSL2 and ZFP57 (Table 3, Fig 5 and S6 Table). CHRNA5 encodes a nicotinic acetylcholine receptor subunit and SNPs in this locus have been associated with body weight in relation to tobacco use [38]. G6PC2 encodes glucose-6-phosphatase catalytic subunit 2 and SNPs in this locus have been associated with glycemic traits [39]. GPX7 encodes glutathione peroxidase 7 a protein involved in glutathione
metabolism. RPL27A encodes Ribosomal protein L27A, which has been linked to human obesity [40]. THNSL2 encodes threonine synthase like 2 and SNPs in this locus have been associated with obesity [41]. Additionally, ZFP57 encodes a zink finger protein and DNA methylation and mutations in this locus are associated with transient neonatal diabetes [42].

The 2,735 unique SNPs identified in the trans-mQTL analysis were also followed-up and related to mRNA expression of all analyzed genes. In the eQTL analysis of significant trans-mQTL SNPs, we identified 270 significant associations between genotypes and mRNA expression levels after correction for multiple testing [see Method], consisting of 89 unique SNPs and 10 unique genes e.g. GSTT1, HLA-DQB1 and ZFP57 (Table 3 and S7 Table).

The impact of identified mQTLs on metabolic phenotypes

Given that adipose tissue contributes to whole body energy homeostasis by for instance insulin-stimulated glucose uptake, triglyceride storage and adipokine secretion, we investigated if the identified SNPs in significant mQTLs affect metabolic phenotypes in our study cohort. Identified mQTL SNPs were related to obesity measurements, glycemic traits and lipid levels in our study cohort of 119 Scandinavian men as well as looked-up in public available consortia data from the GIANT [43,44], MAGIC [36,45,46] and GLGC [47] consortia. Out of the significant cis-mQTLs, we found 62 SNPs associated with BMI, 185 with waist-hip ratio (WHR), 77 with fasting glucose, 62 with fasting insulin, 91 with homeostasis model of beta-cell function (HOMA-B), 49 with HOMA-IR, 146 with HbA1c, 85 with total cholesterol, 84 with triglycerides, 197 with HDL, 67 with LDL in both our study cohort and consortia data with the same direction of allele effects and with P \leq 0.05 (S8 Table). Several of these SNPs show genome-wide significance in GIANT, MAGIC or GLGC. Representative associations between genotype and some metabolic traits as well as DNA methylation in the 119 Scandinavian men are shown in Fig 6A–6C. The SNPs presented in Fig 6 do also show genome-wide significance with respective trait in GLGC (rs2523453, cholesterol, p = 6.5 \times 10^{-08} and rs7205804, HDL, p = 5.27 \times 10^{-675}) and MAGIC (rs11603334, fasting glucose, p = 2.9 \times 10^{-08}), respectively (S8 Table).

Table 3. Number of significant eQTL results in human adipose tissue.

|                      | eQTLs of cis-mQTL-SNPs | eQTLs of trans-mQTL-SNPs |
|----------------------|------------------------|--------------------------|
| SNP-mRNA transcript pairs | 926                    | 270                      |
| Unique SNPs          | 635                    | 89                       |
| Unique mRNA transcripts | 101                   | 14                       |
| Unique genes         | 86                     | 10                       |

Only SNPs of significant mQTLs are included in the eQTL analysis. SNPs of significant cis-mQTLs are regressed against mRNA expression of mRNA transcripts located in cis (\leq 500kb). SNPs of significant trans-mQTLs are regressed against mRNA expression of all mRNA transcripts. Significance threshold < 0.05 after correction for multiple testing. Correlation value for eQTL analysis for cis-mQTL-SNPs = 934,021. Correlation value for eQTL analysis for trans-mQTL-SNPs = 33,326,082.
Fig 5. mQTLs affect gene expression in human adipose tissue. Significant mQTL SNP-CpG pairs where the SNP also shows significant association with gene expression in adipose tissue. The boxplots represent some identified mQTL SNPs and associations of the same loci with mRNA expression: (a) CHRNA5, (b) G6PC2, (c) GPX7, (d) RPL27A, (e) THNSL2 and (f) ZFP57. Annotations for these mQTLs are included in S1 Table.

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Fig 6. mQTLs in human adipose tissue affect metabolic phenotypes. The boxplots show significant mQTL SNPs associated with metabolic phenotypes in our study cohort with p < 0.05, and associations of these loci with DNA methylation in adipose tissue for (a) rs2523453, (b) rs7205804, (c) rs11603334.

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Additionally, 8 loci detected in the overlap of the cis-mQTL and eQTL analysis were among those associated with metabolic phenotypes (S8 and S9 Tables). These data show the effect of interactions between common genetic variation and DNA methylation on gene expression and metabolic outcome (depiction presented in Fig 7A–7E, all overlapping SNPs presented in S9 Table).

Of identified trans-mQTLs, we found 2 SNPs associated with BMI, 13 with WHR ratio, 6 with fasting glucose, 2 with fasting insulin, 6 with total cholesterol, 7 with triglycerides, 68 with HDL, and 4 with LDL in both our study cohort and consortia data with the same direction of allele effects and with P ≤ 0.05 (S10 Table).

Additionally, 2 of the identified cis-mQTL SNPS were previously found to be associated with C-reactive protein (CRP) levels (S11 Table).

Additionally, some of the identified cis- and trans-mQTLs are annotated to candidate genes for adipose-related traits. Out of the 157 loci previously implicated in lipid biology in GLGC consortium [47], 48 (30%) were found among 5,589 unique genes annotated to significant cis-mQTLs (S1 Table), and 4 among 375 unique genes annotated to significant trans-mQTLs (S2 Table).

Causality inference test (CIT)—DNA methylation potentially mediates the genetic impact on metabolic phenotypes

We proceeded to evaluate the potential causality relationships between genotypes (G), DNA methylation (M) and phenotypic traits (P) using the CIT [21]. The possible relationships between these three factors are shown in Fig 8. The CIT was performed in our cohort of 119 Scandinavian men for identified SNP-CpG pairs in the mQTL analysis where the SNP also showed significant association with a metabolic phenotype in both our study cohort and publicly available consortia data with P ≤ 0.05. For cis-mQTLs, we identified 39 SNP-CpG pairs, corresponding to 35 unique SNPs and 22 unique CpGs, where SNP plays a causal role on metabolic phenotype, mediated by DNA methylation (Table 4). Out of these 39 SNP-CpG pairs, 1 pair was significantly associated with BMI, 2 for fasting glucose, 1 for fasting insulin, 1 for HOMA-B, 7 for HOMA-IR, 7 for HbA1c, 9 for cholesterol, 1 for triglycerides 5 for HDL and 5 for LDL (Table 4). Among the genes annotated to these SNP-CpG pairs, CDK2API, HLA-DMA, MCM6, TCF19, CAMK1D and NEIL2 were found. None of the cis-mQTLs showed a reactive relationship between a SNP and a metabolic phenotype.

Biological replication of mQTLs in human adipose tissue

To validate whether the results of mQTL analysis hold in an independent cohort, we also looked for overlap with a recent study also showing associations between genetic variation and DNA methylation in human adipose tissue [16]. While both studies analyzed DNA methylation using the Infinium HumanMethylation450 BeadChip, Grundberg et al. restricted their mQTL analysis to SNPs located within 100 kb from analyzed CpG sites [16] and therefore it was only possible to compare some of our results. It should also be taken into account that while our study included men, the study by Grundberg et al. included women and the two studies used different bioinformatic and statistical approaches, which may affect the possibility to replicate the results. Nevertheless, among our significant cis-mQTLs, we found that 5,468 CpG sites also stand under genetic control of SNPs in the study by Grundberg et al., and out of these 2,118 (38.6%) were associated with the same SNP in both their and our study [16].

Additionally, we recently performed an mQTL analysis in human pancreatic islets [20]. Here, we looked for overlap between the significant mQTLs identified in human adipose tissue of the 119 men and the mQTLs previously found in human pancreatic islets [20]. Among our
significant cis-mQTLs in adipose tissue, 39,386 were also found in pancreatic islets (S12 Table). Moreover, 1,852 significant trans-mQTLs overlapped between the two different tissues (S13 Table).

mQTLs in human adipose tissue do also show differential DNA methylation in patients with type 2 diabetes

We have previously identified CpG sites that are differentially methylated in adipose tissue from subjects with type 2 diabetes compared with non-diabetic controls [15]. However, it remains unknown if methylation of these sites may also be under genetic control. Therefore, we further tested if these CpG sites [15] overlap with our significant cis and trans mQTLs in human adipose tissue (S1 and S2 Tables). Interestingly, we discovered that 237 CpG sites among our significant cis-mQTLs and 7 CpG sites among our significant trans-mQTLs are also differentially methylated in adipose tissue from subjects with type 2 diabetes (S14 Table), suggesting that DNA methylation may mediate the genetic impact of type 2 diabetes.

mQTLs in human adipose tissue overlap with CpG sites associated with BMI and HbA1c

We have previously identified CpG sites for which the adipose tissue methylation level associates with BMI and HbA1c [48]. Here, we examined if these CpG sites overlap with our cis and trans-mQTLs in human adipose tissue (S1 and S2 Tables). We found that 33,058 CpG sites previously identified as associated with BMI overlapped with 577 cis and 19 trans significant mQTLs in current study (S15 Table). Moreover, out of 711 CpG sites associated with HbA1c, 25 and 1 CpG site overlapped with significant cis and trans mQTLs respectively (S15 Table).

mQTL analyses in adipose tissue of two sub-cohorts

Since the subjects in the four sub-cohorts included in this study differ in age and BMI, we performed a sub-analysis only including cohorts #1 and #2 as these subjects are phenotypically similar. Here, we detected 66,329 mQTLs in cis showing significant associations between genotype and the degree of DNA methylation after correction for multiple testing, corresponding to

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**Fig 7. mQTLs/eQTLs in human adipose tissue affect metabolic phenotypes.** Significant mQTL SNPs associated with both gene expression and a metabolic phenotype, with boxplots showing associations of some of these loci with DNA methylation, gene expression and metabolic traits: (a) rs619824, (b) rs7349405, (c) rs7210728, (d) rs176095, (e) rs529488.

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**Fig 8. CIT in human adipose tissue.** Possible relationship models between genotype as a causal factor (G), DNA methylation as the mediator factor (M) and metabolic phenotype as the phenotypic outcome (P).

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| Chr | CpG Id   | CpG Gene   | CpG Gene Region | SNP Id     | SNP Gene   | G vs M pcorr-value | Phenotype          | G vs P p-value | CIT causal p-value |
|-----|----------|------------|-----------------|------------|------------|---------------------|--------------------|---------------|-------------------|
| 6   | cg12929486 | SLC22A16   | TSS200          | rs2428190  | SLC22A16   | 1.30E-07            | BMI               | 0.049         | 0.02             |
| 5   | cg14825688 | LEAP2      | TSS1500         | rs39830    | UQCRQ      | 4.02E-06            | Fasting glucose    | 0.015         | 0.03             |
| 5   | cg14825688 | LEAP2      | TSS1500         | rs803217   | -          | 5.20E-05            | Fasting glucose    | 0.018         | 0.03             |
| 2   | cg01726273 | -          | Intergenic      | rs4853438  | SNRPG      | 3.60E-02            | Fasting insulin    | 0.050         | 0.03             |
| 8   | cg11123440 | Intergenic | rs12458         | GATA4      |            | 8.99E-03            | HOMA-B             | 0.042         | 0.03             |
| 12  | cg10240950 | C12orf76   | Body            | rs1027949  | GIT2       | 3.91E-02            | HOMA-IR            | 0.036         | 0.05             |
| 12  | cg10240950 | C12orf76   | Body            | rs10774978 | TCHP       | 3.91E-02            | HOMA-IR            | 0.030         | 0.05             |
| 12  | cg10240950 | C12orf76   | Body            | rs1168884  | GIT2       | 3.91E-02            | HOMA-IR            | 0.017         | 0.05             |
| 10  | cg26169081 | CAMK1D;    | Body;Body       | rs11257926 | CAMK1D     | 1.33E-03            | HOMA-IR            | 0.021         | 0.04             |
| 10  | cg26169081 | CAMK1D;    | Body;Body       | rs17152029 | CAMK1D     | 3.92E-04            | HOMA-IR            | 0.009         | 0.01             |
| 10  | cg26169081 | CAMK1D;    | Body;Body       | rs17152037 | CAMK1D     | 3.92E-04            | HOMA-IR            | 0.009         | 0.01             |
| 12  | cg10240950 | C12orf76   | Body            | rs2302689  | ANKRD13A   | 3.91E-02            | HOMA-IR            | 0.030         | 0.05             |
| 7   | cg17372657 | Intergenic | rs1880296       |            |            | 2.69E-07            | Hba1c              | 0.032         | 0.03             |
| 7   | cg17372657 | Intergenic | rs2949170       |            |            | 1.79E-04            | Hba1c              | 0.034         | 0.03             |
| 7   | cg17372657 | Intergenic | rs2949192       |            |            | 9.02E-05            | Hba1c              | 0.014         | 0.03             |
| 6   | cg13561028 | SFTA2      | Body            | rs3130782  | LOC100129065 | 3.68E-07            | Cholesterol        | 0.013         | 0.04             |
| 6   | cg13561028 | SFTA2      | Body            | rs3131934  |            | 8.49E-08            | Hba1c              | 0.002         | 0.04             |
| 16  | cg04544033 | ARL6IP4;   | TSS1500;3'UTR   | rs1084689  | CDK2AP1    | 4.91E-03            | Cholesterol        | 0.013         | 0.04             |
| 12  | cg21745287 | ARL6IP4;   | TSS1500;3'UTR   | rs1084689  | CDK2AP1    | 2.34E-02            | Cholesterol        | 0.013         | 0.04             |
| 12  | cg21745287 | ARL6IP4;   | TSS1500;3'UTR   | rs1109559  |            | 4.84E-03            | Cholesterol        | 0.011         | 0.03             |
| 12  | cg07644039 | ARL6IP4;   | TSS1500;3'UTR   | rs1109559  |            | 1.44E-02            | Cholesterol        | 0.011         | 0.03             |
| 12  | cg07644039 | ARL6IP4;   | TSS1500;3'UTR   | rs4275659  | ABCB9      | 2.24E-03            | Cholesterol        | 0.010         | 0.02             |
| 12  | cg21745287 | ARL6IP4;   | TSS1500;3'UTR   | rs6488868 | SBN01      | 9.39E-04            | Cholesterol        | 0.008         | 0.02             |
| 15  | cg12371991 | Intergenic | rs6494591       |            |            | 8.73E-03            | Cholesterol        | 0.038         | 0.01             |
| 2   | cg04490207 | Intergenic | rs6712567       |            |            | 1.15E-02            | Cholesterol        | 0.034         | 0.03             |
| 9   | cg14341289 | FSD1L      | TSS1500         | rs885954   |            | 1.81E-02            | Cholesterol        | 0.041         | 0.00             |
| 8   | cg00820056 | Intergenic | rs11787024      | LYBH       |            | 6.37E-03            | Triglycerides      | 0.033         | 0.04             |
| 6   | cg14833385 | HLA-DMA    | TSS1500         | rs1490380  |            | 3.50E-17            | HDL                | 0.007         | 0.03             |
| 2   | cg01726273 | Intergenic | rs2921711       | TIA1       |            | 3.60E-02            | HDL                | 0.037         | 0.04             |
| 2   | cg07169764 | MCM6;MCM6  | 1stExon;5'UTR   | rs309172   | DARS       | 4.06E-07            | HDL                | 0.011         | 0.01             |
| 8   | cg05875700 | ERICH1     | Body            | rs3735917  | ERICH1     | 3.17E-11            | HDL                | 0.008         | 0.05             |
| 2   | cg07169764 | MCM6;MCM6  | 1stExon;5'UTR   | rs6750549  | DARS       | 4.06E-07            | HDL                | 0.010         | 0.01             |
| 4   | cg08020340 | MYL5       | Body            | rs11726338 | PIGG       | 9.60E-03            | LDL                | 0.028         | 0.04             |
| 6   | cg02525939 | Intergenic | rs4710698       |            |            | 6.17E-07            | LDL                | 0.007         | 0.04             |
| 6   | cg04399728 | Intergenic | rs4710698       |            |            | 2.69E-13            | LDL                | 0.007         | 0.04             |

(Continued)
36,909 unique SNPs and 11,788 unique CpG sites (S16 Table). Out of those 66,329 mQTLs, 63,714 (96%) overlapped with the analysis of all 4 cohorts.

In the trans-mQTL analysis, we identified 3,243 SNP-CpG pairs showing significant associations between genotypes and the degree of DNA methylation in adipose tissue after correction for multiple testing, corresponding to 1,865 unique SNPs and 538 unique CpG sites (S17 Table). Out of those 3,243 mQTLs, 2,919 (90%) were previously identified in the analysis of all 4 cohorts.

mQTL analyses in adipose tissue without adjusting for BMI

In order to validate whether BMI as a covariate has a significant effect on a number of discovered mQTLs, we performed a mQTL analysis of all 4 study cohorts without BMI as a covariate. Overall, we detected 102,467 significant cis mQTLs corresponding to 51,435 unique SNPs and 15,267 unique CpG sites. Out of those, 99,661 (97.2%) were also identified in the analysis where BMI was included as a covariate (S18 Table). In trans, we discovered 5,435 significant mQTLs, corresponding to 608 unique CpG sites and 2,765 unique SNPs, where 5,272 (97%) were also identified in the main mQTL analysis (S19 Table).

Associations between DNA methylation and mRNA expression in human adipose tissue

We finally tested the direct association between DNA methylation and gene expression in human adipose tissue by performing a linear regression between individual mRNA transcripts and DNA methylation of CpG sites in cis (500 kb up- and 100 kb downstream of respective gene) including age, BMI and study cohort as covariates. We found significant associations between DNA methylation and mRNA expression for 546 combinations (FDR < 5%), consisting of 473 unique CpG sites and 194 unique mRNA transcripts (S20 Table), which are annotated to 173 genes.

In addition, we found that 262 CpG sites among our significant cis-mQTLs and 13 among our significant trans-mQTLs overlapped with methylation sites associated with mRNA expression in adipose tissue (S20 Table).

Discussion

The present study highlights the importance of genome-wide interactions between genetic and epigenetic variation and its role in human metabolism. Using CIT tests, we could for the first time identify adipose tissue methylation-mediated relationships between genotype and metabolic phenotypes, including lipid and glucose traits. Importantly, these data demonstrate how genetic variants may mediate their effects on metabolic traits via altered DNA methylation in human adipose tissue. Additionally, numerous identified mQTL-SNPs cover previously identified GWAS loci for obesity, lipid and diabetes related traits e.g. POMC, GIPR, GRB10, FADS2, SORT1 and APOA5.
Multiple SNPs identified through GWAS associate with complex metabolic disease including obesity and type 2 diabetes [29,31,43,49–52]. However, the effect sizes of common variants influencing these diseases are often modest and in total they only explain small proportions of the estimated genetic predispositions to the diseases. Epigenetic factors such as DNA methylation have also been shown to be involved in the pathogenesis of various metabolic diseases [7,9,15,29,53–60]. However, studies examining the genetic regulation of inter-individual variation in DNA methylation and its contribution to metabolic outcomes are scarce but would likely give new insights to the field. Here, we performed a genome-wide mQTL analysis looking at both cis and trans effects of genetic variation on DNA methylation in human adipose tissue. To further link identified mQTLs with biological functions, we performed follow-up analyses of significant mQTL SNPs with gene expression in adipose tissue and metabolic phenotypes in our study cohort. We also looked for overlap with disease loci reported to associate with obesity and diabetes related traits in GWAS. All together, we found 101,911 SNP-CpG pairs in cis and 5,342 SNP-CpG pairs in trans showing significant associations between genotype and DNA methylation in adipose tissue demonstrating a strong genetic impact on DNA methylation in human adipose tissue. Our data are in line with previous mQTL analyses, which also show strong interactions between genetic and epigenetic variation [16–20,61], and in concordance, we found an enrichment of cis-mQTLs in a short distance window between associated SNPs and CpG sites. However, while most previous mQTLs have been limited to studying promoter regions [17–19] or cis interactions [16], we can for the first time present mQTL results in adipose tissue looking at both cis and trans effects in most genomic regions and genes. Interestingly, we observe a higher than expected number of methylation sites in significant mQTLs located in intergenic regions, in the gene body and outside of CpG islands. This observation is in line with previous studies showing that differentially methylated sites in response to environmental or genetic factors to a higher extent than expected are located outside CpG islands or within intergenic and gene body regions [10,16]. It may be that promoter regions are rich in CpG islands which are hypomethylated and are more evolutionary conserved based on their biological function, meanwhile non-CpG islands are more methylated and dynamic [6,62–64]. Interestingly, we demonstrate for the first time an enrichment of significant mQTLs in adipose tissue on chromosome 6. This chromosome possesses a highly polymorphic gene region coding the HLA complexes which are known to be implicated in several autoimmune disorders and inflammation processes [23,24]. Numerous loci identified in the cis- and trans-mQTL analysis, as well as genes in the eQTL follow-up analysis, are linked to the HLA genes. Based on this finding, we investigated the link between mQTLs on chromosome 6 and a measure of inflammation e.g. i.e. CRP levels. Interestingly, we found that 2 SNPs in significant mQTLs cover GWAS loci associated with CRP levels (S11 Table). However, none of them was located on chromosome 6 [65].

Genetic association studies have improved our understanding of the biological basis of metabolic disease [66]. Nevertheless, the effect of numerous reported obesity and diabetes SNPs on target genes or biology still remains unknown. Investigating the genetic control of variation in DNA methylation may improve our understanding of biological processes and linking loci to tissue dependent phenotypes and diseases. Elevating, we found that several SNPs associated with DNA methylation show impact on metabolic phenotypes in the studied cohort, including obesity measurements, glucose- and insulin traits, as well as lipid profiles. The effect of mQTLs on molecular phenotypes was further supported by independent replication in consortia data of obesity measurements from GIANT [43,44], glucose traits from MAGIC [36,45,46] and lipid profiles from GLGC [47]. Although mQTL SNPs were only showing nominal association to metabolic phenotypes in our study cohort, the overlap and replication in independent studies, based on consortium data, support effects of these SNPs on biological function. Indeed,
several of these SNPs show genome-wide significance in previous GWAS [47,66–68]. These include SNPs associated with cholesterol levels and annotated to ANKRD31 (ankyrin repeat domain 31), HDL levels and annotated to CELSR2 (cadherin, EGF LAG seven-pass G-type receptor 2) as well as fasting plasma glucose levels and annotated to ARAP1 (ankyrin repeat and PH domain 1).

Given that SNPs affect DNA methylation and that DNA methylation is a dynamic process that may change in response to environmental factors and affects phenotype transmission [10,69], it may be possible that the SNP effect on DNA methylation levels, and indirectly on metabolic phenotypes, may change under different environmental conditions. It is hence possible that some of the identified mQTL SNPs overlapping with consortium data may have escaped detection to disease phenotypes in previous GWAS studies since DNA methylation levels was not considered. This form of gene-environment interactions could potentially affect the SNPs impact on disease risk. Indeed, our previous data, where we identified a SNP that introduces a CpG site in the promoter of NDUFB6, support this hypothesis [60]. Here, we showed that while elderly carriers of the genotype that introduces a CpG site had a high degree of methylation in the SNP-CpG site together with decreased skeletal muscle NDUFB6 expression and decreased glucose uptake, young carriers had low degree of methylation in the SNP-CpG site together with increased skeletal muscle NDUFB6 expression and no effect on glucose uptake. Together, this study demonstrates a clear interaction between genetic, epigenetic and non-genetic factors. Additionally, genetic variation may carry inheritance of epigenetic variation and thereby have an impact on the heritability of human diseases and may explain some of the missing heritability of human complex diseases. Furthermore, we also found that several SNPs associated with DNA methylation in adipose tissue overlapped directly or via proxy SNPs to previously reported disease loci of obesity related traits, including CETP and FADS2, which are both known to be associated with total cholesterol, LDL, HDL and triglyceride levels [47] These data support that genetic and epigenetic variation together influence metabolic phenotypes and disease risk in humans.

In order to provide further insights into mechanisms of genetic and epigenetic interaction and its impact on regulation of metabolic phenotypes, we used the CIT [21,70]. We discovered 39 significant mQTLs where DNA methylation represents the mediator between genetic loci and a metabolic trait. One of these mQTLs SNPs is associated with HDL regulation through DNA methylation of a CpG site annotated to MCM6. This is an MCM (minichromosome maintenance) complex gene that previously has been shown to affect total cholesterol levels [71]. Among other genes identified in the CIT analysis were TCF19, which has been associated with type 1 diabetes through GWAS [72], and CAMK1D, which has been associated with type 2 diabetes [73] This supports the role of DNA methylation as a direct mediator between genetic variation and metabolic phenotypes. However, while the majority of significant cis-mQTL SNP-CpG pairs were found to have independent effects on the analyzed phenotypes, the independence cannot be concluded due to some limitations in our analysis. First, only a few phenotypes were considered in the course of the analysis, and it might require other phenotypes to discover all cause–effect relationships between SNPs, methylation and metabolic phenotype. Second, as CIT only considers one SNP and one CpG site at a time, more complex interactions involving several SNPs and or CpGs can be missed, which suggests that more sophisticated analytical methods should be developed. An additional drawback of our study is the small sample size relative to the number of statistical comparisons. As the number of analyzed SNP–CpG pairs is in the order of $10^{11}$, only the strongest interaction effects can be detected by means of our mQTL analysis. To reduce the number of type 2 errors during multiple testing procedures, we implemented a modified Bonferroni correction method, which took into account a linkage disequilibrium dependency between analyzed SNPs. Additionally, we performed eQTL and
CIT analysis only on the data that was shown to be significant in the mQTL analysis, thus again significantly reducing number of performed statistical tests. While eQTL analyses have been used to identify causal genetic variants for metabolic disease [74], here we provide the first CIT analysis of genetic variation, DNA methylation in adipose tissue and metabolic traits. Importantly, this analysis demonstrates how genetic variants mediate their effects on metabolic traits (e.g. BMI, cholesterol, HDL, HbA1c and HOMA-IR) via altered DNA methylation in human adipose tissue.

Interestingly, SNPs throughout the genome may introduce or delete CpG sites and thereby affect the possibility for DNA methylation to take place [22]. These so called CpG-SNPs are likely to show strong correlations with the degree of methylation in the SNP site. Indeed, here we found 447 CpG-SNPs associated with DNA methylation in adipose tissue.

Furthermore, we were able to replicate numerous of our unique CpG sites of significant cis-mQTLs in a study by Grundberg et al. [16] confirming the biological importance of our results. While both our study and Grundberg et al. performed mQTL analyses in human adipose tissue using the Illumina 450K array for DNA methylation and thereby comparable, divergence in cis boundary, sex and correction methods for multiple testing may explain some of the different results between the studies. It should also be noted that 39,386 of our significant cis-mQTLs in human adipose tissue were previously also identified in human pancreatic islets [20]. While this finding shows that some SNPs affect the DNA methylation pattern in multiple tissues, additional mQTL studies using the 450k array are needed in other tissues to test if the same associations are seen there.

We provide for the first time a combined genome-wide cis- and trans-mQTL analysis in human adipose tissue covering most genes and genomic regions. Our study demonstrates that interactions between genetic and epigenetic variation influences gene expression, molecular phenotypes and metabolic traits related to complex diseases in humans. We also provide details on potential causal relationships between genetic and epigenetic variation on metabolic phenotypes. Thus, DNA methylation variation may be of high importance in genetic association studies and may improve our understanding of molecular pathways in the context of complex human metabolic diseases.

**Materials and Methods**

**Study samples and phenotypes**

This study includes a total of 119 Scandinavian men without known disease. Their characteristics are presented in Table 1. The cohort includes subjects from four sub-cohorts, all previously described [15,48,75–77] and with DNA available from subcutaneous adipose tissue biopsies taken in the fasted state. The characteristics of the four sub-cohorts are presented separately in S21 Table. All study participants underwent a physical examination including measurements of BMI, waist and WHR. Moreover, blood sampling for analysis of lipids, glucose and insulin were done during the fasting state. Written informed consent was obtained from all participants and the research protocols were approved by the local human research ethics committees: Dnr 13/2006 (Lund University), Dnr 461/2006 (Lund University), KA 03129gm (Køpenhavns AMT). While three of sub-cohorts are intervention studies [75–77], one sub-cohort is a case-control cohort [15]. Only baseline samples from healthy subjects were included in this study.

**Genotype data**

Genotyping was performed in DNA extracted from blood of the 121 Scandinavian men using Illumina HumanOmniExpress BeadChip, which is a genome-wide array covering 731,412
SNPs, together with the iScan system (Illumina, San Diego, CA, USA). Genomic DNA was extracted from blood using the Gentra Puregene Blood Kit (Qiagen, Hilden, Germany). Genotypes were called using GenomeStudio software (Illumina). All subjects passed call rate threshold of > 98%. Sex discrepancy between reported sex and genotypic sex based on X-chromosome heterozygosity was detected for two subjects and these subjects were excluded from subsequent analyses. No subjects were found to be population outliers based on a population stratification test. SNPs were excluded if missing calls > 5%, Hardy-Weinberg Equilibrium p-value < 0.001 and minor allele frequency < 0.05. Overall, 592,794 SNPs for 119 subjects passed quality control and were used for subsequent analyses. All genotype data were analyzed using Plink software (http://pngu.mgh.harvard.edu/purcell/plink/) [78].

DNA methylation data

Genome-wide DNA methylation profiling was performed in genomic DNA extracted using Qiagen DNA extraction kits (Qiagen) from adipose tissue from 119 Scandinavian men using the Infinium HumanMethylation450 BeadChip (Illumina). The DNA methylation array targets 485,577 probes across the genome, covering 99% of RefSeq genes and 96% of CpG islands. Genomic DNA (500 ng) from adipose tissue was bisulfite treated using the EZ DNA methylation kit (Zymo Research, Orange, CA, USA). DNA methylation analysis of bisulfite treated DNA was carried out with Infinium assay following the standard Infinium HD Assay Methylation Protocol Guide (Part #15019519). BeadChips were scanned with Illumina iScan and raw data was imported to the GenomeStudio Methylation module software for calculation of methylation scores represented as methylation β-values. In sample quality control, all samples passed GenomeStudio quality control steps for bisulfite conversion efficiency, staining, hybridization, extension and specificity.

Individual probes with a mean Illumina detection p-value > 0.01 were considered not detected and subsequently excluded from further analysis. Non-CpG methylation probes and SNP-probes included on the array were also filtered out. After these quality control steps and after filtering DNA methylation data, 477,891 CpG sites remained for all included samples. Before further analysis, the DNA methylation data was exported from GenomeStudio and subsequently analyzed using Lumi package from Bioconductor [79]. Extracted methylation data were then converted from β-values to M-values [80], $M = \log_2 \left( \frac{\max(M) + 1}{\min(U) + 1} \right)$, where M and U are methylated and unmethylated channel intensities, respectively. The data was further background corrected and quantile normalized using lumi package [81]. To correct for batch effects, COMBAT normalization method [82] was used.

mRNA expression data

Genome-wide mRNA expression profiling using the whole-transcript GeneChip Human Gene 1.0 ST Array (Affymetrix, Santa Clara, CA, USA) following the Affymetrix standard protocol was performed in RNA extracted from the subcutaneous adipose tissue biopsies of 118 out of 119 Scandinavian men using miRNeasy kit followed by the RNeasy MiniElute Cleanup Kit (Qiagen) or using the RNeasy Lipid Tissue Mini Kit (Qiagen). The array data was background corrected, quantile normalized and summarized with robust multichip average (RMA) procedure using oligo package [83] from Bioconductor. Normalized dataset was batch corrected using COMBAT [82]. In total, mRNA expression of 28,779 transcripts was obtained for subsequent analyses.
mQTL analysis

Associations between SNPs and DNA methylation of CpG sites were modeled as a linear relationship using DNA methylation levels as a dependent variable, SNP genotypes encoded as 0, 1 or 2 according to number of minor alleles. Due to the fact that both BMI and age can affect DNA methylation and, therefore, the association between SNP and DNA methylation, age, BMI and the sub-cohort were included as covariates. Calculations of associations were performed using the MatrixEQTL library for R programming language [84].

To distinguish between local (cis) and distant (trans) mQTLs a distance less or equal to 500 kb between a SNP and CpG site was used to define cis-mQTLs. All remaining SNP-CpG pairs were considered trans-mQTLs. In total we found 283,290,917,454 CpG–SNP pairs in the dataset, where 112,842,462 pairs were defined to be located in cis and 283,178,074,992 in trans. The cis- and trans-mQTL analyses were performed separately. In order to correct for multiple testing, p-value significance threshold was set, accounting for number of tests performed as well as the dependency of linkage disequilibrium (LD) between SNPs. LD-based SNP pruning was used to take into account the linkage dependency of SNPs that are run against the same quantitative trait locus in the mQTL analysis by calculating the number of independent tests based on \( r^2 < 0.9 \) for the SNPs. In the cis-analysis, LD based pruning of SNPs within a distance of 500 kb from a CpG site was performed by pairwise-tagging \( (r^2 < 0.9) \) and the total sum of all tag SNPs connected to each CpG site was used as correction value when correcting for multiple testing. LD calculations were performed using R trio package [85]. The correction value for the trans-analysis was calculated as the total number of analyzed CpG sites multiplied by the number of tag SNPs in the whole dataset (pairwise-tagging \( r^2 < 0.9 \)) and subtracted by the correction value for the cis-analysis. Significance threshold was set to \( p < 0.05 \) after correction for multiple testing. All SNPs connected to each CpG site after LD-based pruning were summed and the remaining number of 104,023,091 SNP-CpG pairs was used as correction value for multiple testing in cis. This resulted in a significance threshold of 0.05/104,023,091 = 4.8x10^{-10} in cis. In the trans-mQTL analysis, after LD-based pruning, 211,781,637,483 SNP-CpG pairs remain and this number was used as correction value for multiple testing. This resulted in a significance threshold of 0.05/211,781,637,483 = 2.3x10^{-13} in trans.

Impact of significant mQTL SNPs on mRNA expression

The relationship between SNPs found to be significantly associated with DNA methylation in the mQTL analysis and mRNA expression was tested in 118 of the men included in the study using a linear regression model with mRNA expression as a dependent variable, SNP genotypes encoded as 0, 1 or 2 according to number of minor alleles, and age, BMI and sub-cohort as covariates. Significant SNPs identified in the cis-mQTL analysis were only related to mRNA expression transcripts of genes located within 500 kb from respective SNP (cis). Significant SNPs identified in the trans-mQTL were related to mRNA expression transcripts of all analyzed genes. In total, 1,164,807 SNP-mRNA transcript combinations were found for significant cis-mQTLs, and 78,710,565 SNP-mRNA transcript combinations were found for significant trans-mQTLs. Correction value for multiple testing in the eQTL analysis was then calculated in similar way as for the mQTL analysis taking LD-based SNP pruning \( (r^2 < 0.9) \) into account. In the eQTL analysis of significant cis-mQTL SNPs, the number of LD pruned SNPs \( (r^2 < 0.9) \) to each mRNA transcript within 500 kb were summed up and used as the correction value for multiple testing. After LD-based pruning, 934,021 SNP-mRNA transcripts remain. This resulted in a significance threshold of 0.05/934,021 = 5.4x10^{-8} in cis. In the eQTL analysis of significant trans-mQTL SNPs, the correction value for multiple testing was calculated as the number of all trans-mQTL SNPs pruned for LD \( (r^2 < 0.9) \) multiplied by total number of
analyzed mRNA transcripts giving a remaining number of 33,326,082 SNP-mRNA transcripts. This resulted in a significance threshold of $0.05/33,326,082 = 1.5 \times 10^{-9}$ in trans.

Impact of mQTL SNPs on metabolic phenotypes

The impact of identified SNPs in significant mQTLs on the following phenotypes; BMI, WHR, cholesterol, triglycerides, HDL, LDL, fasting glucose, fasting insulin, HOMA-B, HOMA-IR and HbA1c, was tested in 119 Scandinavian men included in this study. Associations between identified SNPs in the significant mQTLs and metabolic phenotypes were modeled as a linear relationship using metabolic phenotypes as the dependent variable, SNP genotypes encoded as 0, 1 or 2 according to number of minor alleles, and age and sub-cohort included as covariates in all the analyses. BMI was also included as a covariate when analyzing associations between SNPs and fasting glucose, fasting insulin, HOMA IR, HOMA-B and HbA1c. Traits for fasting insulin, HOMA-B and HOMA-IR have been naturally log transformed in the study cohort before analyses. Identified mQTL SNPs showing association to a metabolic phenotype in our study cohort ($p < 0.05$), were also looked-up in public available GWAS data from the GIANT consortium [43,44], MAGIC investigators [36,45,46] and GLGC consortium [47], for respective trait. SNPs showing association to a metabolic phenotype with the same allelic effect sign and with $p$-value $< 0.05$ in both our study cohort and consortia data were considered detected.

Overlap between mQTL SNPs and public available GWAS data

The catalog of published GWAS data was used to search for SNPs reported to be associated with obesity, type 2 diabetes and related metabolic traits ($p < 10^{-5}$). To increase reference coverage for overlap between datasets of identified mQTL SNPs and identified SNPs reported in GWAS catalog, a SNP annotation and proxy (SNAP) search [86] was performed to identify SNPs in LD with the identified mQTL SNPs. The proxy search was based on pairwise LD calculations of genotype data from the 1000 Genomes project of the CEU population panel with $r^2 > 0.8$ and distance limit of 500 kb from the query SNP.

Causal Inference Test (CIT)

The CIT was used to test if DNA methylation is a mediator between genotype variation and a phenotypic trait [21]. The causality can be inferred if all of the following are true: 1) G and M are associated, 2) G and P are associated, 3) G is associated with M|P and 4) G is independent of P|M, where G is a genotype marker, M is a DNA methylation measure and P is a phenotypic trait, provided that G is randomized [21]. Causal role of DNA methylation is inferred if $p$-value for causal relationship hypothesis is less than 0.05.

Statistical analysis

Data were analyzed using linear regression models, Pearson chi-squared test or Fisher’s exact test. All statistical calculations were performed using R programming language [87]. Results are expressed as Box and Whiskers plots. Pathway analysis using WebGestalt [26].

Supporting Information

S1 Table. Identified cis-mQTLs. Sheet a: Identified cis-mQTL SNP-CpG pairs, including chromosomal location and relation to CpG islands and gene regions. Sheet b: SNP-CpG pairs where SNP is located in either C or G of the CpG site, so called CpG-SNPs. Sheet c: Additional annotation data for SNPs present in sheet a, based on HumanOmniExpress-12v1_I_Gene_Annotation_build37 (Illumina). Sheet d: Additional annotation data for CpGs present in sheet a,
based on Infinium HumanMethylation 450 BeadChip [25] and probe cross-reactivity info as reported by Chen et al [88].

**S2 Table. Identified trans-mQTLs.** Sheet a: Identified trans-mQTL SNP-CpG pairs, including statistical results of associations and chromosomal location and relation to CpG islands and gene regions. Sheet b: Additional annotation data for SNPs present in sheet a, based on HumanOmniExpress-12v1_J_Gene_Annotation_build37 (Illumina). Sheet c: Additional annotation data for CpGs present in sheet a, based on Infinium HumanMethylation 450 BeadChip [25] and probe cross-reactivity info as reported by Chen et al [88].

**S3 Table. KEGG pathways identified among genes annotated to significant cis mQTL CpG sites.** Sheet a: KEGG pathways enriched among genes annotated to CpG sites from significant cis-mQTLs. Sheet b: KEGG pathways enriched among genes annotated to CpG sites from significant trans-mQTLs.

**S4 Table. Identified cis-mQTL SNPs that are also reported as disease SNPs in GWAS catalog [27].** SNPs that are found directly in the catalog are marked with grey, and ones that are found to be in LD with GWAS catalog SNPs with white. LD proxy analysis performed using SNAP (1000 Genomes project, CEU population panel, r2 > 0.8, distance limit 500kb) [86].

**S5 Table. Identified trans-mQTL SNPs that are also reported as disease SNPs in GWAS catalog [27].** SNPs that are found directly in the catalog are marked with grey, and ones that are found to be in LD with GWAS catalog SNPs with white. LD proxy analysis performed using SNAP (1000 Genomes project, CEU population panel, r2 > 0.8, distance limit 500kb) [86].

**S6 Table. Identified significant cis-mQTL SNPs that also show associations with gene expression.** Sheet a: Identified cis SNP-mRNA transcript pairs, including statistical results of associations and gene assignment for mRNA transcripts. Only pairs with p-value < 0.05 after multiple testing corrections are included. Sheet b: Additional annotation data for SNPs present in sheet a, based on HumanOmniExpress-12v1_J_Gene_Annotation_build37 (Illumina). Sheet c: Additional annotation data for probesets present in sheet a. Annotations are based on NetAffx transcript cluster data for HuGene-1_0-st array (Affymetrix).

**S7 Table. Identified significant trans-mQTL SNPs that also show associations with gene expression.** Sheet a: Identified trans SNP-mRNA transcript pairs, including statistical results of associations and gene assignment for mRNA transcripts. Sheet b: Additional annotation data for SNPs present in sheet a. Sheet c: Additional annotation data for probesets present in sheet a. Annotations are based on NetAffx transcript cluster data for HuGene-1_0-st array (Affymetrix).

**S8 Table. Identified cis-mQTL SNPs that show association with metabolic phenotypes in the study cohort (p<0.05) and are also identified in MAGIC, GIANT, or GLGC consortia (p<0.05).** Excel table representing cis-mQTL SNPs that are identified in MAGIC, GIANT, or GLGC consortia (p<0.05) overlapping with SNPs that show association with metabolic phenotypes in the study cohort (p<0.05). Sheet a: SNPs associated with BMI in study cohort and
GIANT consortium [44]. Sheet b: SNPs associated with Waist-hip ratio in study cohort and
GIANT consortium [43]. Sheet c: SNPs associated with Fasting glucose in study cohort and
MAGIC consortium [45]. Sheet d: SNPs associated with Fasting insulin in study cohort and
MAGIC consortium [45]. Sheet e: SNPs associated with HOMA-B in study cohort and
MAGIC consortium [45]. Sheet f: SNPs associated with HOMA-IR in study cohort and
MAGIC consortium [45]. Sheet g: SNPs associated with HbA1c in study cohort and MAGIC
consortium [46]. Sheet h: SNPs associated with Total cholesterol in study cohort and GLGC
consortium [47]. Sheet i SNPs associated with Triglycerides in study cohort and GLGC consor-
tium [47]. Sheet j: SNPs associated with HDL in study cohort and GLGC consortium [47].
Sheet k: SNPs associated with LDL in study cohort and consortium GLGC [47].

S9 Table. Significant cis-mQTL/eQTL SNPs that show association with metabolic pheno-
types in the study cohort and are also identified in MAGIC, GIANT, or GLGC consortia
(p<0.05).

S10 Table. Identified trans-mQTL SNPs that show association with metabolic phenotypes
in the study cohort (p<0.05) and are also identified in MAGIC, GIANT, or GLGC consor-
tia (p<0.05). Sheet a: SNPs associated with BMI in study cohort and GIANT consortium [44].
Sheet b: SNPs associated with Waist-hip ratio in study cohort and GIANT consortium [43].
Sheet c: SNPs associated with Fasting glucose in study cohort and MAGIC consortium [45].
Sheet d: SNPs associated with Fasting insulin in study cohort and MAGIC consortium [45].
Sheet e: SNPs associated with HOMA-B in study cohort and MAGIC consortium [45]. Sheet f:
SNPs associated with HOMA-IR in study cohort and MAGIC consortium [45]. Sheet g: SNPs
associated with HbA1c in study cohort and MAGIC consortium [46]. Sheet h: SNPs associated
with Total cholesterol in study cohort and GLGC consortium [47]. Sheet i SNPs associated
with Triglycerides in study cohort and GLGC consortium [47]. Sheet j: SNPs associated with
HDL in study cohort and GLGC consortium [47]. Sheet k: SNPs associated with LDL in study
cohort and consortium GLGC [47].

S11 Table. Significant cis-mQTL SNPs associated with CRP in Denghan et al. [65].

S12 Table. Significant cis-mQTL SNP-CpG pairs that are also reported to show significant
associations in Olsson et al. [20].

S13 Table. Significant trans-mQTL SNP-CpG pairs that are also reported to show signifi-
cant associations in Olsson et al. [20].

S14 Table. Significant cis- and trans- mQTL CpG sites that are also reported to show differ-
ential methylation in Nilsson et al. [15]. Sheet a: Identified cis-mQTL CpG sites that are
reported in Nilsson et al. [15]. Sheet b: Identified trans-mQTL CpG sites that are reported in
Nilsson et al. [15]. Sheet c: Additional annotation data for CpGs present in sheet a, based on
Infinium HumanMethylation 450 BeadChip. [25]. Sheet d: Additional annotation data for
SNPs present in sheet a, based on HumanOmniExpress-12v1_I_Gene_Annotation_build37
(Illumina).
S15 Table. Significant *cis-* and *trans-* mQTL CpG sites that are also reported to show significant associations with BMI and HbA1c in Rönn et al. [48].

(SLSX)

S16 Table. *cis*-mQTLs identified in the analysis of subcohorts 1 and 2. Sheet a: Identified *cis-*mQTL SNP-CpG pairs, including chromosomal location and relation to CpG islands and gene regions. Sheet b: SNP-CpG pairs where SNP is located in either C or G of the CpG site, so called CpG-SNPs. Sheet c: Additional annotation data for SNPs present in sheet a, based on HumanOmniExpress-12v1_I_Gene_Annotation_build37 (Illumina). Sheet d: Additional annotation data for CpGs present in sheet a, based on Infinium HumanMethylation 450 BeadChip [25] and probe cross-reactivity info as reported by Chen et al [88].

(SLSX)

S17 Table. *trans*-mQTLs *cis-*mQTLs identified in the analysis of subcohorts 1 and 2. Sheet a: Identified *trans-*mQTL SNP-CpG pairs, including statistical results of associations and chromosomal location and relation to CpG islands and gene regions. Sheet b: Additional annotation data for SNPs present in sheet a, based on HumanOmniExpress-12v1_I_Gene_Annotation_build37 (Illumina). Sheet c: Additional annotation data for CpGs present in sheet a, based on Infinium HumanMethylation 450 BeadChip [25] and probe cross-reactivity info as reported by Chen et al [88].

(SLSX)

S18 Table. *cis*-mQTLs identified without BMI as a covariate. Sheet a: Identified *cis-*mQTL SNP-CpG pairs, including chromosomal location and relation to CpG islands and gene regions. Sheet b: SNP-CpG pairs where SNP is located in either C or G of the CpG site, so called CpG-SNPs. Sheet c: Additional annotation data for SNPs present in sheet a, based on HumanOmniExpress-12v1_I_Gene_Annotation_build37 (Illumina). Sheet d: Additional annotation data for CpGs present in sheet a, based on Infinium HumanMethylation 450 BeadChip [25] and probe cross-reactivity info as reported by Chen et al [88].

(SLSX)

S19 Table. *trans*-mQTLs identified without BMI as a covariate. Sheet a: Identified *trans-*mQTL SNP-CpG pairs, including statistical results of associations and chromosomal location and relation to CpG islands and gene regions. Sheet b: Additional annotation data for SNPs present in sheet a, based on HumanOmniExpress-12v1_I_Gene_Annotation_build37 (Illumina). Sheet c: Additional annotation data for CpGs present in sheet a, based on Infinium HumanMethylation 450 BeadChip [25] and probe cross-reactivity info as reported by Chen et al [88].

(SLSX)

S20 Table. Association between DNA methylation and gene expression in human adipose tissue.

(SLSX)

S21 Table. Sample characteristics of 4 different cohorts included in the study.

(SLSX)

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Author Contributions

Conceived and designed the experiments: PV AHO CL. Performed the experiments: L. Gillberg EN TR. Analyzed the data: PV AHO CL. Contributed reagents/materials/analysis tools: SWJ CB PAJ K-FE L. Groop AV. Wrote the paper: PV AHO CL.

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