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Numerous Genes in Loci Associated With Body Fat Distribution Are Linked to Adipose Function

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Central fat accumulation is a strong risk factor for type 2 diabetes. Genome-wide association studies have identified numerous loci associated with body fat distribution. The objectives of the current study are to examine whether genes in genetic loci linked to fat distribution can be linked to fat cell size and number (morphology) and/or adipose tissue function. We show, in a cohort of 114 women, that almost half of the 96 genes in these loci are indeed associated with abdominal subcutaneous adipose tissue parameters. Thus, adipose mRNA expression of the genes is strongly related to adipose morphology, catecholamine-induced lipid mobilization (lipolysis), or insulin-stimulated lipid synthesis in adipocytes (lipogenesis). In conclusion, the genetic influence on body fat distribution could be mediated via several specific alterations in adipose tissue morphology and function, which in turn may influence the development of type 2 diabetes.

Adipose tissue is distributed in many different body regions. Central body fat accumulation is governed by genetic factors (1) and is, independently of total adiposity, associated with metabolic conditions such as type 2 diabetes and cardiovascular disease (2). A recent genome-wide association (GWA) meta-analysis identified 68 genetic loci that associate with body fat distribution, primarily waist-to-hip ratio (WHR) but also related traits such as waist circumference (3). Bioinformatics analysis of the genes in the vicinity of the 68 genetic loci implicated adipose pathways in the regulation of fat distribution, and several of the candidate genes are indeed expressed at the mRNA level in adipose tissue. However, direct evidence for these loci and/or genes being involved in adipocyte function is lacking in most cases.

Fat mass is determined by adipose tissue morphology (i.e., size and number of adipocytes) (4), and both parameters contribute to the total weight of specific human fat depots, thereby regulating fat distribution (5). In addition, alterations in adipocyte function can also influence adipose distribution. The lipid droplet constitutes >95% of the fat cell volume. As reviewed (6), impaired ability of the major lipolysis-regulating hormones, catecholamines, to stimulate the hydrolysis of adipocyte lipids (lipolysis) may cause retention of lipids in fat cells and thereby contribute to fat mass expansion. Insulin is a major anabolic hormone and controls lipid accumulation in fat cells. The ability of insulin to stimulate fat synthesis through glucose conversion into lipids (lipogenesis) could therefore also be of importance. Here, we investigated whether these adipocyte phenotypes (adipose morphology, lipolysis, and lipogenesis) could be linked to abdominal subcutaneous white adipose tissue (WAT) expression of 96 candidate genes in the vicinity of the recently described genetic loci for body fat distribution (3). This centrally located adipose region contributes to variations in WHR and in other parameters of body fat distribution.

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RESEARCH DESIGN AND METHODS

The study included 114 Swedish women without diabetes who were recruited from the general adult population in the Stockholm (Sweden) area (Table 1). They displayed a large interindividual variation in BMI and WHR. The study was approved by the regional ethics board, and written informed consent was obtained from each subject. They were investigated in the morning after an overnight fast, when abdominal subcutaneous WAT was obtained by fine-needle aspiration (7). WAT gene expression was assessed by Gene 1.0 or 1.1 ST Affymetrix arrays, as described (8). Between-array results were normalized in Affymetrix Power Tools.

Adipocytes isolated from abdominal subcutaneous WAT were used to measure activation of lipolysis by isoprenaline (a synthetic catecholamine) and insulin-stimulated lipogenesis, as described (9), and data distribution is summarized in Table 1. By comparing the size of the adipocytes with the total amount of body fat, the morphology of WAT could be quantitatively determined (Δ values), as described in detail (10). Negative Δ values indicate hyperplasia (many small adipocytes), and positive Δ values indicate hypertrophy (few but large adipocytes); the distribution of values is reported in Table 1. Adipose hypertrophy is linked to insulin resistance and the development of type 2 diabetes, as discussed (10).

Linear regression analysis showed age did not influence the examined parameters. As expected, an inverse relationship was found between BMI and insulin-stimulated lipogenesis (r = 0.27) or catecholamine-stimulated lipolysis (r = 0.28, $P \leq 0.004$ for both) but, in accordance with previous studies (10), not with adipose morphology (r = 0.18, P = 0.13). Therefore, further analyses on lipolysis and lipogenesis were adjusted for BMI.

Table 1—Characteristics of 114 women

	Mean	Range
Age, years	43	22–72
BMI, kg/m^2	34	20–53
WHR	0.93	0.74–1.09
WAT morphology, pL	19	−400 to +494
Isoprenaline activation of lipolysis, ratio	8.7	1.4–66.5
Insulin stimulated lipogenesis, nmol of glucose/2 h/10^7 adipocytes	4.71	0.12–46.19

Fat cells were isolated from adipose tissue and incubated in vitro, exactly as described (8), to determine lipolysis (glycerol release) and lipogenesis (incorporation of radiolabeled glucose into fat cell lipids). The cells were incubated in the absence or presence of increasing concentrations of isoprenaline (a synthetic catecholamine) or insulin, and the results at maximum effective concentrations were used. The isoprenaline effect was measured as the ratio of glycerol release without the drug. On a separate aliquot of isolated fat cells, their mean volume was measured and set in relation to the total body fat content, exactly as described in detail (10), to obtain an adipose tissue morphology value. The measured volume was compared with the calculated expected volume given the subject's total fat mass. The difference between measured and calculated fat cell volume is the morphology value.

RESULTS

First, we analyzed all 22,168 probe sets on the arrays for association with WHR and the adipose phenotypes in R software using the SAM Bioconductor package (11). Among significant genes, we focused on GWA candidates for body fat distribution; that is, all genes that were localized within 250 kb of the lead single nucleotide polymorphism in the loci associated with body fat distribution listed by Shungin et al. (3) in their Tables 1, 2, and 3. This included all primary candidate genes in 49 genetic loci associated with WHR adjusted for BMI (WHRadjBMI) in the aforementioned GWA study (3). We also included additional candidate genes that were obtained by expression quantitative trait loci (eQTL) analysis or given high priority according to bioinformatics analysis and all primary candidate genes in 19 loci associated with waist and hip circumference measures. Three genes were not represented with probes on the Affymetrix microarrays. The remaining 96 genes are listed in Supplementary Table 1. The subset of genes whose expression was associated with WHR and with adipose phenotypes according to SAM analysis is shown in Supplementary Table 2.

We next extended the analysis to take into account possible confounders. To this end, multiple regression analysis was used to investigate the associations between the expression of the 96 genes and WHR or the adipocyte phenotypes, taking into account the use of different array batches and, when appropriate, BMI. Because 96 genes were investigated, we considered a P value of < 0.0005 as statistically significant. There was a strong overlap between the results obtained by SAM and those obtained by multiple regression, although somewhat more genes were significant with SAM. We therefore focused on results obtained by regression analysis, which takes potential confounders into account.

As observed in Table 2, only two genes—*CALCRL* and *FAM13A*—were correlated with WHR and WHRadjBMI, which is in accordance with previous WAT expression data on a few of the WHR-associated genes (12). *CALCRL* and *FAM13A* were negatively associated with adipose morphology, whereas *CPEB4*, *HLA-DRA*, *MSC*, and *PLCG2* correlated positively with this phenotype. Thus, WHR-associated genes may influence adipocyte size and number in abdominal subcutaneous WAT in opposite ways, resulting in different body fat distribution. Eight candidate genes for body fat distribution in Table 2 correlated with catecholamine-stimulated lipolysis, where all but one (*CBX3*) showed a positive association. None of these genes associated with adipose morphology, indicating that some genes linked to body fat distribution may influence central fat accumulation by specifically enhancing (in most cases) or suppressing the lipolytic activity of adipocytes.

Table 2—Relationship between WAT gene expression and adipose morphology, isoprenaline-stimulated activation of lipolysis in adipocytes, or WHR

	WAT morphology		Lipolysis		WHR	
Gene ID	Standard coefficient	*P* value	Standard coefficient	*P* value	Standard coefficient	*P* value
CALCRL§□	−0.44	<0.0001	—	—	−0.39	<0.0001
CBX3	—	—	−0.77	0.0001	—	—
CPEB4§	0.59	<0.0001	—	—	—	—
EYA2	—	—	0.43	<0.0001	—	—
FAM13A§¤	−0.46	<0.0001	—	—	−0.49	<0.0001
FGF2#	—	—	—	—	−0.65	<0.0001
HLA-DRA§	0.36	0.0003	—	—	0.41	<0.0001
HMGXB4	—	—	0.56	0.0002	—	—
MSC	0.57	<0.0001	—	—	—	—
PLCG2	0.60	0.0002	—	—	—	—
PPARG#	—	—	0.76	<0.0001	—	—
PTPDC1	—	—	0.53	<0.0001	—	—
SFXN2	—	—	0.61	<0.0001	—	—
TBX15#	—	—	0.56	<0.0001	—	—
VPS53	—	—	0.69	0.0005	—	—

Multiple regression analysis was used with array batch and BMI (the latter only for lipolysis and WHR) as cofactors. For adipose morphology, negative standard coefficient values indicate a stimulatory effect of gene expression on hyperplasia and positive values indicate a stimulatory effect on hypertrophy. Lipolysis values were 10-log transformed. $P \leq 0.0005$ was considered to be statistically significant because 96 genes on the arrays were investigated in total. §Only expression data in adipose tissue reported in the literature. #Functional data in adipocytes/adipose tissue reported in the literature. □Also significantly correlated with WHRadjBMI, which is an adjustment described before (3).

As demonstrated in Table 3, a surprisingly large number of genes correlated with insulin-stimulated lipogenesis, 14 genes in a positive fashion and 21 in an inverse fashion. Apparently, regulation of insulin-induced lipid synthesis (stimulation or inhibition) in adipocytes is a very important pathway in WAT that may link candidate genes to central fat distribution. Similar to lipolysis and morphology, the lipogenesis-associated genes seem specific for this adipocyte process because only 4 (*CBX3*, *HLA-DRA*, *PLCG2*, and *PTPDC1*) overlapped with the 15 genes associated with the other examined adipose phenotypes. These genes, linked to insulin function, may have a particular importance for the type 2 diabetes risk associated with central fat accumulation because insulin resistance is a key feature in this diabetic condition.

We used three batches of arrays to determine gene expression; however, results were similar within each of the batches. Combining the Gene 1.0 and 1.1 ST Affymetrix array results had a negligible effect on the findings. Data in Tables 2 and 3 were validated by real-time quantitative PCR (Supplementary Table 3) using RNA from subcutaneous WAT obtained from a previously examined cohort of 55 women (9). We chose five genes displaying among the strongest correlations in the aforementioned tables and examined the relationship between WAT morphology, WHR, or insulin-stimulated lipogenesis and gene expression. Seven of the eight examined relationships showed a statistically significant relationship in agreement with the data presented in Tables 2 and 3; the eighth relationship was directionally consistent in the validation cohort.

To further explore the function of genetic variants associated with body fat distribution, we performed *cis*-eQTL analysis of the genes in Tables 2 and 3. We related WAT expression of 32 genes to the genotype of 26 single nucleotide polymorphisms representing different genetic loci. We confirmed eQTLs for *ZNF664* (rs4765219) and *CMIP* (rs2925979) and identified a new eQTL for *PDXDC1* (rs4985155) (Supplementary Table 4) (3), three genes whose expression is associated with insulin-stimulated lipogenesis. The limited number of significant associations might be due to limited statistical power because only data from ~100 subjects were available for eQTL analysis.

We finally investigated the expression pattern of the genes in Tables 2 and 3 during differentiation of human subcutaneous progenitor cells to adipocytes (Table 4) using previously published gene expression array data (9). There was no clear pattern for genes associated with lipolysis or lipogenesis (values not shown). However, five of the six morphology-linked genes in Table 1 showed a significant up- or down-regulation during differentiation *(CPEB4*, *PLCG2*, *HLA-DRA*, *FAM13A*, and *MSC)*. This indicates that some genes associated with body fat distribution may

Table 3—Relationship between WAT gene expression and insulin-stimulated lipogenesis in abdominal subcutaneous adipocytes

Gene ID	Standard coefficient	*P* value	Gene ID	Standard coefficient	*P* value
ARL15§	−1.11	<0.0001	*NUDT6*	−0.71	0.0002
BCL2#	0.58	0.0005	*NKX2–6*	0.92	<0.0001
CBX3	0.98	<0.0001	*PDXDC1*§	−0.49	0.0004
CEP250	0.67	<0.0001	*PIGC*§	0.75	0.0001
CMIP§	−0.86	<0.0001	*PLCG2*	−0.72	<0.0001
DNM3§	−0.62	0.0002	*PLXND1*#	−1.12	<0.0001
FGF2#	0.71	<0.0001	*PTPDC1*	−0.51	0.0001
FGFR4#	0.96	<0.0001	*RFX7*	−0.99	<0.0001
GMDS	−0.80	<0.0001	*SFXN2*	−0.57	0.0003
GORAB	−0.61	<0.0001	*SPATA5*	−0.71	<0.0001
GRB14#	−0.39	<0.0001	*TBX15*#	−0.55	0.0002
HLA-DRA§	0.38	0.0003	*TFPI*§	−0.82	<0.0001
HOXA11	0.62	<0.0001	*UQCC*	0.38	0.0001
ITGB6	0.77	<0.0001	*VPS53*	−0.89	<0.0001
KIAA1731	−0.73	<0.0001	*WARS2*§	−0.60	<0.0001
KLF13	−0.56	0.0003	*ZBTB7B*	0.63	<0.0001
KLF14#	0.61	<0.0001	*ZNF664*§	0.54	0.0001
MAP3K1#	−0.51	<0.0001	—	—	—

Multiple regression was used with array batch and BMI as cofactors. Lipogenesis values were 10-log transformed. See legend to Table 2 for further details. §Only expression data in adipose tissue are reported in the literature. #Functional data in adipocytes/adipose tissue are reported in the literature.

influence WAT morphology via effects on adipose differentiation.

DISCUSSION

When data are taken together, 45 of 96 of the genes associated with genetic loci for body fat distribution show a relationship between WAT gene expression and different aspects of adipocyte function linked to subcutaneous abdominal fat accumulation. Most of these genes were associated with one specific adipocyte phenotype (morphology, lipolysis, or lipogenesis), which points to a very distinct role for each gene. Thus, our findings suggest a central role for adipocytes in linking genetic variants with central fat mass distribution. Admittedly, only a few genes showed correlation with WHR. One plausible explanation is that fat distribution is the far away clinical end point of multiple pathways partly differing between subjects (i.e., a multifactorial etiology involving heterogeneity between subjects). Specific genes can then show a stronger association with intermediate phenotypes than with the clinical outcome. It should also be clarified that the genetic association between WHR and the genetic loci studied here becomes strong when very large numbers of individuals are included. Because our present cohort included only 114 subjects, it is quite expected that the association with WHR becomes nonsignificant.

What is known about the role of the examined genes in fat cells? For each of the genes presented in Tables 2 and 3, we searched for previous publications related to the topic on PubMed (http://www.ncbi.nlm.nih.gov/pubmed). We used the key words “adipocyte,” “fat cell,” “adipose tissue,” “lipolysis,” and “lipogenesis.” The genes

Table 4—Expression of genes associated with adipose morphology during adipocyte differentiation in 12 subjects

Gene	Probe set	Day 4	Day 8	Day 12	Day 12 vs. 4	*P* (day 4 vs. 12)
CPEB4	8110055	162 ± +22	229 ± +73	273 ± +97	1.69	0.0020
PLCG2	7997453	45 ± +11	32 ± +10	36 ± +13	0.80	0.0160
HLA-DRA	8118548	351 ± +210	490 ± +367	601 ± +479	1.71	0.0210
FAM13A	8101728	185 ± +56	244 ± +73	264 ± +128	1.43	0.0370
MSC	8151334	494 ± +208	296 ± +107	321 ± +153	0.65	0.0440
CALCRL	8057578	60 ± +49	90 ± +45	86 ± +41	1.43	0.1500

Values are mean + SD. Expression on day 4 vs. 12 was compared by paired *t* test.

were categorized as follows: no publications relevant for the key words, expression data available, or functional data available. The bioinformatics analysis revealed that the mechanism of action on adipocytes is unknown and/or that only gene expression data are available for most of the described genes (Tables 2 and 3). Therefore, the molecular and causal aspects need to be investigated much further. Earlier GWA studies suggest important sex dimorphism for WHR-associated genetic loci (13,14). Because we only studied women, the possible influence of sex remains to be established. Finally, the functional analyses were performed on adipocytes isolated from the centrally located subcutaneous fat. Thus, the effect of the examined gene loci on visceral, gluteal, or other WAT regions with regard to metabolic function or morphology is not known at present. Unfortunately, it is not possible for ethical reasons to study deep fat depots in the present clinical setting. Nevertheless, our findings support the hypothesis that the genetic influence on body fat distribution in humans is governed to a large extent governed by the morphology and metabolic function of adipocytes. Whether this also involves the endocrine role of adipocytes remains to be established.

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