Identification of differentially expressed genes in subcutaneous adipose tissue from subjects with familial combined hyperlipidemia

Petra M. H. Eurlings,* Carla J. H. van der Kallen,* Jan M. W. Geurts,* Paul Kouwenberg,† Willy D. Boeckx,† and Tjerk W. A. de Bruin[1],*

Cardiovascular Research Institute Maastricht,* Laboratory of Molecular Metabolism and Endocrinology, Department of Internal Medicine, University of Maastricht, Maastricht, The Netherlands; and Department of Plastic Surgery,† Academic Hospital Maastricht, The Netherlands

Abstract Subjects with familial combined hyperlipidemia (FCHL) are characterized by a complex metabolic phenotype with hyperlipidemia, insulin resistance, and central obesity. FCHL is due to impaired adipose tissue function superimposed on hepatic overproduction of lipoproteins. We investigated adipose tissue as an interesting target tissue for differential gene expression in FCHL. Human cDNA expression array analyses, in which adipose tissue from five FCHL patients was compared with that from four age, gender, and BMI matched controls, resulted in the identification of 22 up-regulated and three down-regulated genes. The genes differentially expressed imply activation of the adipocyte cell cycle genes. Furthermore, the differential expression of the genes coding for tumor necrosis factor α, interleukin 6, and intracellular adhesion molecule 1 support a role for adipose tissue in insulin resistance in FCHL subjects. The observed changes represent a primary genetic defect, an adaptive response, or a contribution of both.—Eurlings, P. M. H., C. J. H. van der Kallen, J. M. W. Geurts, P. Kouwenberg, W. D. Boeckx, T. W. A. de Bruin. **Identification of differentially expressed genes in subcutaneous adipose tissue from subjects with familial combined hyperlipidemia. *J. Lipid Res.* 2002.** 43: **930–935.**

Supplementary key words atlas human cDNA expression array • TNFα • IL-6 • cell cycle genes

Familial combined hyperlipidemia (FCHL; MIM144250) is the most common genetic hyperlipidemia in humans. FCHL is characterized by familial clustering of multiple type hyperlipidemia, with clinical manifestations of premature coronary heart disease (CAD), i.e., before the age of 60. Although FCHL was delineated about 25 years ago, the complex genetics and metabolic phenotypes in FCHL are not fully understood at present (1–7). Hypercholesterolemia, hypertriglyceridemia, elevated plasma apolipoprotein B (apoB), and apoC-III concentrations are found in FCHL (8). Studies have shown abnormalities in lipoprotein metabolism of FCHL patients, including hepatic hypersecretion of apoB containing lipoproteins and delayed clearance of atherogenic triglyceride-rich lipoprotein remnants, such as VLDL remnants (intermediate density lipoproteins) and chylomicron remnants (8, 9). Increased hepatic VLDL secretion contributes to elevated plasma triglycerides (TG), apoB, total cholesterol (TC), and LDL (10). The forces behind the increased hepatic lipoprotein secretion have not been identified yet.

Defects in adipose tissue function are believed to contribute to the FCHL phenotype, but the exact mechanisms are poorly understood. Expression of the FCHL phenotype has been shown to depend, in part, on body mass index (BMI), a marker of adipose tissue mass (11). In addition, FCHL patients have an abnormal FFA metabolism resulting in reduced clearance of FFA from the circulation in the postprandial state (12). FCHL patients are also characterized by impaired insulin-mediated glucose uptake in peripheral tissues, such as muscle, and impaired insulin-mediated removal of plasma FFA (13–15). It has been shown also that adipocytes as well as fibroblasts from FCHL patients exhibit an impaired response to the activity of the acylation-stimulating protein that stimulates the synthesis of TG in peripheral tissues (16). High levels of FFA in the circulation can lead to a decrease in insulin-stimulated glucose uptake in skeletal muscle, as well as an increase in hepatic lipoprotein synthesis (17, 18).

Based on these observations, we investigated adipose tis-

Abbreviations: apo, apolipoprotein; BMI, body mass index; CAD, coronary artery disease; CCDN1, G1/S-specific cyclin D1; CCDN2, G1/S-specific cyclin D2; CDKN1A, cyclin-dependent kinase inhibitor 1A; ERBB3, v-erb-b2 erythroblastic leukemia viral oncogen homolog 3; FCHL, familial combined hyperlipidemia; GADD45, DNA damage-inducible gene GADD45; ICAM1, intracellular adhesion molecule 1; ID1, DNA binding protein inhibitor ID1; IFNGR2, interferon gamma receptor 2; IL-6, interleukin 6; KRBH, Krebs-Ringer bicarbonate; TC, total cholesterol; TG, triglycerides; TNFα, tumor necrosis factor alpha.

[1] To whom correspondence should be addressed.
e-mail: tdb@sint.azm.nl

sue as an interesting target tissue for differential gene expression in FCHL using human cDNA expression arrays. Such differentially expressed genes represent good candidate genes or provide information about signaling pathways that are involved in FCHL adipocyte dysfunction. The primary data showed a consistently different gene expression pattern in FCHL adipose tissue compared with that from age, gender, and BMI matched controls. In total, 25 differentially expressed genes were identified in FCHL adipose tissue.

MATERIALS AND METHODS

Subjects

FCHL probands and controls were recruited through the Lipid Clinic of the Maastricht University Hospital. FCHL families were ascertained as previously described (19). Briefly, FCHL probands had a primary hyperlipidemia with varying phenotypic expression including (untreated) fasting plasma cholesterol (TC) >6.5 mM (250 mg/dl) and fasting plasma triglyceride (TG) concentration >2.3 mM (200 mg/dl), and a positive family history of premature CAD (i.e., before the age of 60 years). In addition, FCHL probands had no xanthomas, no apoE2/E2 genotype, and normal thyroid-stimulating hormone concentrations. Obesity (BMI >30) or diabetes were exclusion criteria for the ascertainment of an FCHL proband (13). The affected FCHL subjects in the present study had each been ascertained as an affected FCHL relative in a FCHL family that contained at least two other first-degree relatives with a cholesterol and/or TG exceeding the above-mentioned diagnostic values. Pedigree analyses showed evidence of the multiple lipoprotein phenotype phenomena, meaning that different relatives show different lipoprotein phenotypes (IIa, IIb, or IV).

The control subjects had a fasting glucose <5.5 mmol/l, fasting TC <6.5 mmol/l, fasting TG < 2.3 mmol/l, and no family history of CAD. The Human Investigation Review Committee of the Academic Hospital Maastricht approved the study protocol and all subjects gave informed consent.

Sample preparation

Four unrelated control subjects and five unrelated FCHL subjects were age, gender, and BMI matched. Four FCHL subjects who used lipid lowering medication had stopped their therapy for 14 days in order to obtain untreated plasma and adipose tissue samples. Three subjects used atorvastatin and one simvastatin as lipid lowering therapy. Before starting the procedure, venous blood was drawn in pre-cooled EDTA (1 mg/ml) tubes after an overnight fast (12–14 h) and prepared by immediate centrifugation for analytical analyses. Plasma lipids and glucose were measured as described before (19, 20). Liposuction samples were taken caudal from the umbilicus under local anesthesia (1% Lidocaine) with a small liposuction cannula (Accelerator III, Byron, CA) (21). From each subject, 3–5 ml adipose tissue was collected and processed immediately. Isolation of adipocytes was based on the method of Rodbell (22), with minor modifications. Within minutes after obtaining the sample, adipose tissue was cut into small fragments and incubated with 2 mg/ml collagenase, followed by gentle shaking in an incubator (humidified, 5% CO_2 and 95% air) at 37°C for 30–60 min in buffer (Krebs-Ringer bicarbonate supplemented with HEPES containing 25 mM $NaHCO_3$, 1.2 mM KH_2PO_4, 4.8 mM KCl, 1.2 mM $MgSO_4$, 118 mM NaCl, 1.25 mM $CaCl_2$, and 10 mM HEPES, pH 7.4) containing 4% BSA. The cell suspension was then filtered through 500 μm nylon mesh and spun at 220 *g* for 1 min to separate stromal vascular cells from mature adipocytes. Isolated adipocytes were washed three times with KRBH supplemented with 0.3% BSA. The purified mature adipocytes were stored in liquid nitrogen until further use.

RNA preparation

Total RNA extraction was performed using the TRIzol reagent (GibcoBRL, Grand Island, NY) according to the manufacturer's protocol. The RNeasy kit (QIAGEN, Germany) was used for total RNA clean up and its quality was assessed following the manufacturer's instructions. In the experiments 2–3.5 μg total RNA was used.

Atlas human cDNA expression array

The Atlas human cDNA array kit was purchased from Clontech Laboraties (Palo Alto, CA). All procedures for probe labeling were accomplished following the manufacturer's recommendations. Probe purification was done using ProbeQuant™ G-50 micro columns (Amersham, NJ) according to the manufacturer's instructions. The probes were denatured at 95°C for 5 min. The membranes were hybridized in ExpressHyb solution overnight at 68°C, washed according to the manufacturer's instructions, and exposed for varying time lengths to an imaging screen (BioRad, Hercules, CA). Stripping of the membranes was carried out according to the manufacturer's instructions and were re-used up to three times. In a typical experiment an FCHL subject was compared with a matched control subject resulting in a total of five experimental pairs.

The intensities of the spots were analyzed using the Quantity One program (BioRad). Every gene was present in duplicate on the array and the average intensity of the duplicate spots was used for analyses. Gene hybridization intensities were determined after background subtraction. The intensities of the spots were corrected for mean background and mean intensities of the housekeeping genes on each array. First, normalized intensities of corresponding spots were compared between the FCHL array and the control array, but only those spots that were visible on the image and did have a homogenous and round shape were taken into account. Differences were expressed as a ratio of normalized spot intensities on the FCHL array and normalized spot intensities on the control array. The expression was considered differential if there was a 2-fold difference in intensity between the FCHL and control array. A ratio ⩾2 indicated up-regulation and a ratio ⩽0.5 indicated down-regulation. Second, genes were considered differentially expressed in FCHL adipose tissue when in 60% or more of the experiments the gene showed differential expression in the same direction, taking only into account those experiments in which the gene was expressed. Third, a minimum of two experiments with differential gene expression was required.

RESULTS

The clinical characteristics of the study subjects are presented in **Table 1**. Venous blood was drawn after an overnight fast and the FCHL patients had stopped their lipid lowering medication for two weeks to ensure expression of their hypercholesterolemia and/or hypertriglyceridemia at the time of the abdominal fat biopsy (Table 1). Three subjects were hypertriglyceridemic and two were hypercholesterolemic. All FCHL subjects had elevated plasma apoB levels >1.2 g/l and were normoglycemic. Controls were normolipidemic and normoglycemic.

TABLE 1. Clinical characteristics study subjects

Trait	FCHL (n = 5)	Control (n = 4)
Male/Female	2/3	1/3
Age (years)	52.4 ± 2.4	50.5 ± 7.6
BMI	25.7 ± 1.4	25.8 ± 1.0
TG (mmol/l)	2.25 ± 0.89	1.26 ± 0.54
TC (mmol/l)	6.4 ± 0.7	4.9 ± 0.69
HDL-C (mmol/l)	0.79 ± 0.13	0.92 ± 0.05
Glucose (mmol/l)	4.94 ± 0.62	5.14 ± 0.24
FFA (mmol/l)	0.35 ± 0.18	0.23 ± 0.25
ApoAI (g/l)	1.46 ± 0.25	1.35 ± 0.07
ApoB (g/l)	1.39 ± 0.16	0.86 ± 0.21

Data represent mean ± SD.

Clontech's Atlas human cDNA expression arrays were used to screen for differential gene expression in FCHL adipose tissue compared with age, gender, and BMI matched controls. These arrays allowed the direct screening of 588 cDNAs, each spotted in duplicate in six functional quadrants. The membranes were hybridized with cDNA samples prepared from 2–3.5 μg total RNA from each FCHL and spouse control adipose tissue sample. Of the 588 genes spotted on a single Atlas expression array, on average 174 (30%) genes showed detectable, i.e., visible, levels of expression, whereas 414 (70%) genes were not expressed at detectable levels in adipocytes. As seen in **Fig. 1**, the average expression ratios from the five experimental pairs have a gaussian distribution, indicating that the arrays provided good quality results. Five of the nine housekeeping genes on the blot were expressed at detectable levels in adipose tissue. Ubiquitin had the highest, and 40S ribosomal protein the lowest, expression level (**Table 2**). As

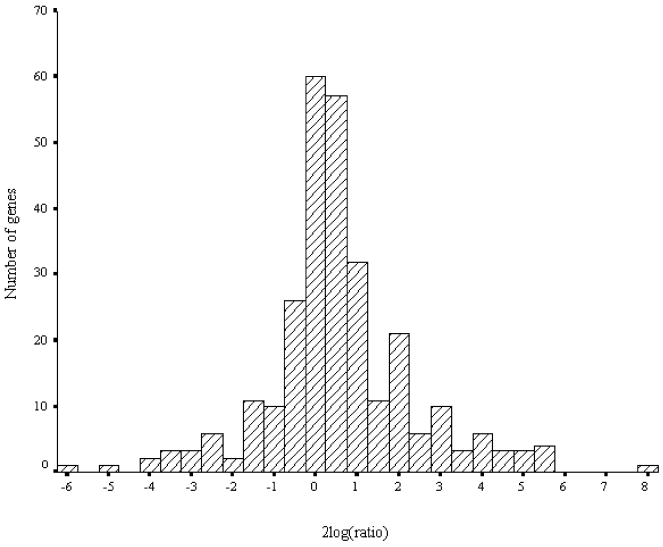

Fig. 1. Quality assessment of the differential gene expression analyses. The average expression ratios of the familial combined hyperlipidemia (FCHL) adipose tissue arrays versus the control adipose tissue arrays are plotted as ^{2}log (ratio). The gaussian distribution of the ratios illustrates that the experiments were of good quality.

TABLE 2. Comparison of the relative housekeeping gene intensities between the FCHL expression arrays and control expression arrays

Housekeeping Gene	FCHL	Spouses
	%	
Ubiquitin	59.8 ± 3.2	64.5 ± 4.2
GAPDH	18.7 ± 3.6	15.2 ± 3.2
ACTB	5.6 ± 2.5	4.4 ± 1.9
23-kDa highly basic protein	13.8 ± 2.6	13.2 ± 1.6
40S ribosomal protein	2.1 ± 0.8	2.7 ± 1.4
Total signal	100	100

Data represent mean ± SD. GAPDH, liver glyceraldehydes 3-phosphate dehydrogenase; ACTB, cytoplasmatic beta-actin.

expected, these housekeeping genes had similar and comparable relative intensities on the FCHL and control expression arrays, indicating that there is little variability in housekeeping gene expression between FCHL adipose tissue and that of controls (Table 2). These data support the reproducibility and reliability of the assay.

In total, 25 differentially expressed genes were identified. **Table 3** lists all genes that fulfilled the criteria for differential expression in FCHL adipose tissue. The data are presented as signal intensities that were equal to, or greater than, 2-fold compared with the intensity of expression on the control array (up-regulation). Alternatively, the data are given as signal intensities that were equal or smaller than 0.5-fold compared with the control array. Table 3 illustrates, for all five experimental pairs, whether a gene is up-regulated, down-regulated, not differentially expressed, or is not expressed at a detectable level. **Fig. 2** shows corresponding segments of an FCHL array and a control array, visually illustrating some of the observed differences in adipose tissue gene expression. It is important to note that the five experiments resulted in the identification of a consistent and uniform genetic fingerprint in FCHL adipose tissue that also showed consistent differences with the gene expression pattern observed in matched spouse controls.

DISCUSSION

In the present study the cDNA macro-array technique was used to identify candidate genes or signaling pathways for (subcutaneous) adipocyte dysfunction in FCHL. It has been suspected that impaired function of adipose tissue can contribute to the FCHL phenotype by inadequate incorporation of FFA into TG in adipocytes (12, 23–25). The resulting increased FFA flux can contribute to increased hepatic lipoprotein production as well as reduced insulin mediated glucose uptake and are both known FCHL phenotypes (17, 23). To our knowledge, we are the first to report on the identification of differentially expressed genes in FCHL adipose tissue using this approach. In total, 25 differentially expressed genes were identified in FCHL adipose tissue, of which 22 were up-regulated and three were down-regulated. This is a first approximation of the differential gene expression in FCHL adipose tissue given the limited number of genes tested.

TABLE 3. Evaluation of genes differentially expressed in FCHL adipose tissue

Gene Name	Genbank ID	Relative Expression Profile in Each FCHL/Control Pair 1	2	3	4	5	FCHL/Control Ratio*
Oncogenes and tumor suppressors							
C-Myc	V00568	+	+	+	+	o	7.6 ± 10.9
C-Jun	J04111	+	o	+	o	+	3.5 ± 3.6
C-Yes	M15990	x	x	x	−	−	0.3 ± 0.3
Adenomatous polyposis coli (APC) protein	M74088; M73548	x	x	+	x	+	8.0 ± 4.7
Cell cycle regulators							
DNA binding protein inhibitor ID1	D13889	+	x	+	x	+	3.2 ± 0.8
G1/S-specific cyclin D1 (CCDN1)	M59798; M64349	+	+	+	o	+	4.2 ± 2.5
G1/S-specific cyclin D2 (CCDN2)	M90813; D13639	+	+	o	−	+	2.8 ± 2.3
Modulators, effectors and intracellular transducers							
Prostaglandin receptor, EP4 subtype (PTGER4)	L25124; D28472	x	+	+	x	x	17.8 ± 17.2
Interferon gamma receptor 2 (IFNGR2)	U05875	x	+	+	x	x	4.8 ± 0.5
Apoptosis-associated proteins							
Tumor necrosis factor alpha (TNFα)	X01394	+	x	+	+	x	13.0 ± 7.6
Fas-activated serine/threonine (FAST) kinase	X86779	x	+	+	x	o	2.0 ± 1.3
Protein inhibitor of neural nitric oxide synthase (PIN)	U32944	+	+	+	o	o	1.7 ± 0.9
DNA-synthesis, repair and recombination proteins							
DNA-damage-inducible transcript 1 (GADD45A)	M60974	x	−	x	x	−	0.04 ± 0.02
Transcription factors and DNA-binding proteins							
CACCC-box DNA-binding protein	L04282	x	−	−	x	−	0.4 ± 0.2
Early growth response protein (EGR1)	X52541; M62829	+	+	+	+	o	6.8 ± 4.7
ETR101	M62831	+	+	+	o	+	2.6 ± 1.4
Cyclin-dependent kinase inhibitor 1A (CDKN1A, p21)	U09579; L25610	+	o	+	+	+	3.8 ± 2.7
Growth factor and chemokine receptors							
Epidermal growth factor receptor (ERBB3)	M29366; M34309	+	o	+	+	+	6.6 ± 8.5
Endothelin receptor type B (EDNRB)	L06623	x	x	+	x	+	9.6 ± 9.5
Cell surface antigens and adhesion proteins							
T-cell activation CD27 antigen	M63928	+	x	+	x	x	20.7 ± 24.0
Intracellular adhesion molecule 1 precursor (ICAM1)	J03132	+	−	+	+	o	229.6 ± 504.4
Cell adhesion protein SQM1	M33374	+	+	+	o	−	4.1 ± 3.2
Growth factors, cytokines and chemokines							
Small inducible cytokine A2 (SCYA2)	M24545	+	+	+	+	o	11.6 ± 10.2
Macrophage inflammatory protein 2 alpha (MIP2)	X53799	+	+	x	+	−	3.2 ± 2.4
Interleukin-6 precursor (IL-6)	X04602; M14584	+	o	+	+	o	2.2 ± 1.4

Relative expression profile of each FCHL/control pair (1 to 5) illustrates whether a gene is up-regulated (+; FCHL/control ratio ⩾ 2.0), down-regulated (−; FCHL/control ratio ⩽ 0.5), not differentially expressed (o; 0.5 > FCHL/control ratio < 2.0), or not expressed at a detectable level (x).

*Data represent mean ± SD.

A large subset of the differentially expressed genes identified in the present study plays a prominent role in cell growth, i.e., c-Myc, c-Jun, G1/S-specific cyclin D1 (CCDN1), G1/S-specific cyclin D2 (CCDN2), DNA binding protein inhibitor ID1, DNA damage-inducible transcript 1 (GADD45), early growth response protein 1, cyclin-dependent kinase inhibitor 1A (CDKN1A), and epidermal growth factor receptor v-erb-b2 erythroblastic leukemia viral oncogen homolog 3 (ERBB3). Each gene product is directly or indirectly involved in the transition of the cell cycle from the G1 to the S phase (26–30). However, the function of these genes in cell cycle regulation of (pre)adipocytes, at least to our knowledge, has not been studied yet. Based upon the observation that c-Myc, c-Jun, CCDN1, CCDN2, ID1, and ERBB3, which stimulate cell cycle transition, are up-regulated in FCHL adipose tissue and that GADD45, which inhibits entry of cells into S phase, is down-regulated, indicate cell cycle gene activation of subcutaneous adipocytes in FCHL subjects most likely due to hyperplasia. The fact that CDKN1A, an inhibitor of G1/S phase transition (31), is up-regulated may be in contrast with these observations, but CDKN1A has also been shown to act as a stimulator of cell growth, and, moreover, its role in adipocytes has not been described. Subcutaneous adipose tissue mass in FCHL is expanded (18); however, it is unknown whether this is due to increased adipocyte cell mass or cell number. We recently reported that BMI, a marker of adiposity, affects the expression of hyperlipidemia in FCHL (11). Continuously increased delivery of lipoproteins and their remnants from plasma to adipocytes in FCHL may increase cell cycle gene expression in order to accommodate the persistent lipid supply. Therefore the subset of cell cycle regulators differentially expressed in FCHL may reflect hyperplasia of adipocytes. This proposed mechanism does not imply that the compensatory hyperplasia of adipocytes is sufficient to maintain normal lipid or FFA metabolism in FCHL.

Impaired insulin action has been reported in FCHL (13–15), and has been linked to altered metabolism of FFA (23–25) and impaired reactivity of adipocyte lipolysis (32). Tumor necrosis factor α (TNFα) gene expression was shown in the present study to be up-regulated in FCHL adipose tissue. It is of interest that the role of TNFα in the development of insulin resistance has been well

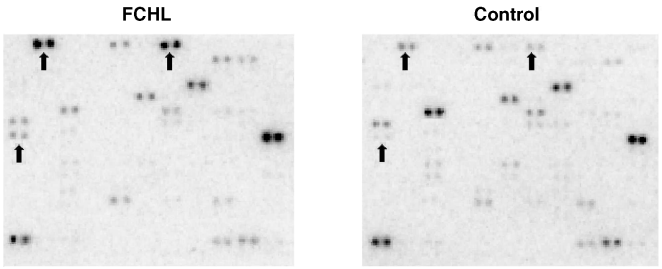

Fig. 2. Gene expression fingerprint of FCHL adipose tissue. The arrows indicate a subset of differentially expressed genes in FCHL adipose tissue compared with control adipose tissue.

documented (33), and a link between the TNFα signaling pathway and FCHL has recently been reported (20, 34). TNFα is known to impair insulin signaling in adipose tissue through inhibition of the insulin receptor tyrosine kinase (35). TNFα also inhibits lipoprotein lipase (LPL) and stimulates lipolysis in adipocytes (36), although there are conflicting data whether LPL activity is affected in FCHL (24, 37). TNFα is also known to induce the synthesis of other proinflammatory molecules such as interleukin 6 (IL-6) (38), and increased plasma IL-6 levels have been associated with insulin resistance (39–41). It is interesting that IL-6 gene expression was clearly up-regulated in FCHL adipose tissue. Additionally, the interferon γ receptor 2 (IFNGR2) was also up-regulated in FCHL adipose tissue, and IFN-γ signaling has been shown to increase plasma IL-6 (42). Interestingly, intracellular adhesion molecule 1 (ICAM1), whose gene expression was also up-regulated in FCHL adipocytes in the present study, has been shown to be involved in insulin resistance through the TNFα system as well (43). Combined, the differential gene expression of TNFα, IL-6, IFNGR2, and ICAM1 in FCHL indicates common denominators in adipose tissue insulin resistance, or the existence of a pro-inflammatory state in FCHL.

The fact that the observed changes in adipose tissue gene expression were consistent among the age, gender, and BMI matched FCHL/control pairs generates new perspectives for future research. Combined with results from FCHL genome-wide screens, gene expression data can help to distinguish primary genetic FCHL defects from adaptive changes in FCHL. Such a genomics approach has proven useful in other complex diseases such as hypertension (44) and diabetes (45), and will be instrumental for FCHL in the near future. The current data cannot distinguish between genes differentially expressed as a result of an adaptive response to hyperlipidemia or a genetic predisposition to FCHL. Therefore, although beyond the scope of the present study, FCHL adipose tissue gene expression should be compared with that of subjects with a different hyperlipidemic disorder such as familial hypercholesterolemia or type II diabetes. On the other hand, in case the observed changes in adipose tissue gene expression are specific for FCHL, it offers the potential to diagnose FCHL with more certainty in individuals without a family study, providing a new tool for genetic and pathophysiological studies.

In conclusion, our results demonstrate a clear and consistent difference in gene expression in FCHL adipose tissue compared with that from matched healthy controls. Some of the genes identified point toward changes in adipose tissue activation of cell cycle genes. Additionally, genes previously described in other insulin resistance states are differentially expressed in FCHL. The present data show that gene expression analyses represent a useful tool to gain more insight into organ specific gene expression in a human complex disease such as FCHL. The question remains whether the observed changes in gene expression represent the results from a primary genetic defect or an adaptive response.

The authors thank the participants for their cooperation with this study. We would also like to thank E. T. P. Keulen, MD, PhD, and J. van Lin for recruitment of FCHL patients and spouses. T.W.A.B. was supported by grant 900.95.297 of the Dutch Organization for Scientific Research (NWO). This study was also supported by CARIM, Cardiovascular Research Institute Maastricht, and the Academic Hospital Maastricht.

Manuscript received 3 August 2001, in revised form 6 March 2002, and in re-revised form 20 March 2002.

REFERENCES

1. Goldstein, J. L., H. G. Schrott, W. R. Hazzard, E. L. Bierman, and A. G. Motulsky. 1973. Hyperlipidemia in coronary heart disease. II. Genetic analysis of lipid levels in 176 families and delineation of a new inherited disorder, combined hyperlipidemia. *J. Clin. Invest.* **52:** 1544–1568.
2. Cullen, P., B. Farren, J. Scott, and M. Farrall. 1994. Complex segregation analysis provides evidence for a major gene acting on serum triglyceride levels in 55 British families with familial combined hyperlipidemia. *Arterioscler. Thromb. Vasc. Biol.* **14:** 1233–1249.
3. Pajukanta, P., I. Nuotio, J. D. Terwilliger, K. V. Porkka, K. Ylitalo, J. Pihlajamaki, A. J. Suomalainen, A. C. Syvanen, T. Lehtimaki, J. S. Viikari, M. Laakso, M. R. Taskinen, C. Ehnholm, and L. Peltonen. 1998. Linkage of familial combined hyperlipidaemia to chromosome 1q21-q23. *Nat. Genet.* **18:** 369–373.
4. Pajukanta, P., J. D. Terwilliger, M. Perola, T. Hiekkalinna, I. Nuotio, P. Ellonen, M. Parkkonen, J. Hartiala, K. Ylitalo, J. Pihlajamaki, K. Porkka, M. Laakso, J. Viikari, C. Ehnholm, M. R. Taskinen, and L. Peltonen. 1999. Genomewide scan for familial combined hyperlipidemia genes in Finnish families, suggesting multiple susceptibility loci influencing triglyceride, cholesterol, and apolipoprotein B levels. *Am. J. Hum. Genet.* **64:** 1453–1463.
5. Aouizerat, B. E., H. Allayee, R. M. Cantor, R. C. Davis, C. D. Lanning, P. Z. Wen, G. M. Dallinga-Thie, T. W. A. de Bruin, J. I. Rotter, and A. J. Lusis. 1999. A genome scan for familial combined hyperlipidemia reveals evidence of linkage with a locus on chromosome 11. *Am. J. Hum. Genet.* **65:** 397–412.
6. Aouizerat, B. E., H. Allayee, R. M. Cantor, G. M. Dallinga-Thie, C. D. Lanning, T. W. A. de Bruin, A. J. Lusis, and J. I. Rotter. 1999. Linkage of a candidate gene locus to familial combined hyperlipidemia–Lecithin: cholesterol acyltransferase on 16q. *Arterioscler. Thromb. Vasc. Biol.* **19:** 2730–2736.
7. Eurlings, P. M. H., C. J. H. van der Kallen, J. M. W. Geurts, M. M. J. van Greevenbroek, and T. W. A. de Bruin. 2001. Genetic dissection of familial combined hyperlipidemia. *Mol. Genet. Metab.* **74:** 98–104.
8. de Graaf, J., and A. F. Stalenhoef. 1998. Defects of lipoprotein metabolism in familial combined hyperlipidaemia. *Curr. Opin. Lipidol.* **9:** 189–196.
9. Castro-Cabezas, M., T. W. A. de Bruin, H. Jansen, L. A. Kock, W. Kortlandt, and D. W. Erkelens. 1993. Impaired chylomicron remnant clearance in familial combined hyperlipidemia. *Arterioscler. Thromb. Vasc. Biol.* **13:** 804–814.

10. Taskinen, M. R., M. J. Caslake, and C. J. Packard. 2001. Lipoprotein metabolism in FCHL. *Eur. J. Clin. Invest.* **31 (Suppl. I):** 14.
11. van der Kallen, C. J. H., R. M. Cantor, M. M. J. van Greevenbroek, J. M. W. Geurts, F. G. Bouwman, B. E. Aouizerat, H. Allayee, W. A. Buurman, A. J. Lusis, J. I. Rotter, and T. W. A. de Bruin. 2000. Genome scan for adiposity in Dutch dyslipidemic families reveals novel quantitive trait loci for leptin, body mass index and soluble tumor necrosis factor receptor superfamily 1A. *Int. J. Obes. Relat. Metab. Disord.* **24:** 1381–1391.
12. Castro-Cabezas, M., T. W. A. de Bruin, H. W. de Valk, C. C. Shoulders, H. Jansen, and D. W. Erkelens. 1993. Impaired fatty acid metabolism in familial combined hyperlipidemia. A mechanism associating hepatic apolipoprotein B overproduction and insulin resistance. *J. Clin. Invest.* **92:** 160–168.
13. Aitman, T. J., I. F. Godsland, B. Farren, D. Crook, H. J. Wong, and J. Scott. 1997. Defects of insulin action on fatty acid and carbohydrate metabolism in familial combined hyperlipidemia. *Arterioscler. Thromb. Vasc. Biol.* **17:** 748–754.
14. Bredie, S. J., C. J. Tack, P. Smits, and A. F. Stalenhoef. 1997. Nonobese patients with familial combined hyperlipidemia are insulin resistant compared with their nonaffected relatives. *Arterioscler. Thromb. Vasc. Biol.* **17:** 1465–1471.
15. Karjalainen, L., J. Pihlajamaki, P. Karhapaa, and M. Laakso. 1998. Impaired insulin-stimulated glucose oxidation and free fatty acid suppression in patients with familial combined hyperlipidemia: a precursor defect for dyslipidemia? *Arterioscler. Thromb. Vasc. Biol.* **18:** 1548–1553.
16. Kwiterovich, P. O., M. Motevalli, and M. Miller. 1994. The effect of three serum basic proteins on the mass of lipids in normal and hyperapoB fibroblasts. *Arterioscler. Thromb. Vasc. Biol.* **14:** 1–7.
17. Lewis, G. F., and G. Steiner. 1996. Acute effects of insulin in the control of VLDL production in humans. Implications for the insulin-resistant state. *Diabetes Care.* **19:** 390–393.
18. Purnell, J. Q., S. E. Kahn, R. S. Schwartz, and J. D. Brunzell. 2001. Relationship of insulin sensitivity and apoB levels to intra-abdominal fat in subjects with familial combined hyperlipidemia. *Arterioscler. Thromb. Vasc. Biol.* **21:** 567–572.
19. Keulen, E. T. P., M. Kruijshoop, N. C. Schaper, A. P. G. Hoeks, and T. W. A. de Bruin. 2002. Increased intima-media thickness in Familial Combined Hyperlipidemia associated with apolipoprotein B. *Arterioscler. Thromb. Vasc. Biol.* **22:** 283–288.
20. Geurts, J. M. W., R. G. J. H. Janssen, M. M. J. van Greevenbroek, C. J. H. van der Kallen, R. M. Cantor, X. Bu, B. E. Aouizerat, H. Allayee, J. I. Rotter, and T. W. A. de Bruin. 2000. Identification of TNFRSF1B as a novel modifier gene in familial combined hyperlipidemia. *Hum. Mol. Genet.* **9:** 2067–2074.
21. Kolaczynski, J. W., L. M. Morales, J. H. Moore, R. V. Considine, Z. Pietrzkowski, P. F. Noto, J. Colberg, and J. F. Caro. 1994. A new technique for biopsy of human abdominal fat under local anaesthesia with Lidocaine. *Int. J. Obes. Relat. Metab. Disord.* **18:** 161–166.
22. Rodbell, M. 1964. Metabolism of isolated fat cells. I. Effects of hormones on glucose metabolism and lipolysis. *J. Biol. Chem.* **239:** 375–380.
23. Meijssen, S., M. Castro-Cabezas, T. B. Twickler, H. Jansen, and D. W. Erkelens. 2000. In vivo evidence of defective postprandial and postabsorptive free fatty acid metabolism in familial combined hyperlipidemia. *J. Lipid Res.* **41:** 1096–1102.
24. Reynisdottir, S., B. Angelin, D. Langin, H. Lithell, M. Eriksson, C. Holm, and P. Arner. 1997. Adipose tissue lipoprotein lipase and hormone-sensitive lipase. Contrasting findings in familial combined hyperlipidemia and insulin resistance syndrome. *Arterioscler. Thromb. Vasc. Biol.* **17:** 2287–2292.
25. Sniderman, A. D., K. Cianflone, P. Arner, L. K. Summers, and K. N. Frayn. 1998. The adipocyte, fatty acid trapping, and atherogenesis. *Arterioscler. Thromb. Vasc. Biol.* **18:** 147–151.
26. Shaulian, E., and M. Karin. 2001. AP-1 in cell proliferation and survival. *Oncogene.* **20:** 2390–2400.
27. Prabhu, S., A. Ignatova, S. T. Park, and X. H. Sun. 1997. Regulation of the expression of cyclin-dependent kinase inhibitor p21 by E2A and Id proteins. *Mol. Cell. Biol.* **17:** 5888–5896.
28. Smith, M. L., I. T. Chen, Q. Zhan, I. Bae, C. Y. Chen, T. M. Gilmer, M. B. Kastan, P. M. O'Connor, and A. J. Fornace. 1994. Interaction of the p53-regulated protein Gadd45 with proliferating cell nuclear antigen. *Science.* **266:** 1376–1380.
29. Sherr, C. J. 1996. Cancer cell cycles. *Science.* **274:** 1672–1677.
30. Lange, C. A., J. K. Richer, T. Shen, and K. B. Horwitz. 1998. Convergence of progesterone and epidermal growth factor signaling in breast cancer. Potentiation of mitogen-activated protein kinase pathways. *J. Biol. Chem.* **273:** 31308–31316.
31. Di-Cunto, F., G. Topley, E. Calautti, J. Hsiao, L. Ong, P. K. Seth, and G. P. Dotto. 1998. Inhibitory function of p21Cip1/WAF1 in differentiation of primary mouse keratinocytes independent of cell cycle control. *Science.* **280:** 1069–1072.
32. Reynisdottir, S., M. Eriksson, B. Angelin, and P. Arner. 1995. Impaired activation of adipocyte lipolysis in familial combined hyperlipidemia. *J. Clin. Invest.* **95:** 2161–2169.
33. Peraldi, P., and B. Spiegelman. 1998. TNF-alpha and insulin resistance: summary and future prospects. *Mol. Cell. Biochem.* **182:** 169–175.
34. van Greevenbroek, M. M. J., C. J. H. van der Kallen, J. M. W. Geurts, R. G. J. H. Janssen, W. A. Buurman, and T. W. A. de Bruin. 2000. Soluble receptors for tumor necrosis factor-alpha (TNF-R p55 and TNF-R p75) in familial combined hyperlipidemia. *Atherosclerosis.* **153:** 1–8.
35. Hotamisligil, G. S., A. Budavari, D. Murray, and B. M. Spiegelman. 1994. Reduced tyrosine kinase activity of the insulin receptor in obesity-diabetes. Central role of tumor necrosis factor-alpha. *J. Clin. Invest.* **94:** 1543–1549.
36. Patton, J. S., H. M. Shepard, H. Wilking, G. Lewis, B. B. Aggarwal, T. E. Eessalu, L. A. Gavin, and C. Grunfeld. 1986. Interferons and tumor necrosis factors have similar catabolic effects on 3T3 L1 cells. *Proc. Natl. Acad. Sci. USA.* **83:** 8313–8317.
37. Babirak, S. P., B. G. Brown, and J. D. Brunzell. 1992. Familial combined hyperlipidemia and abnormal lipoprotein lipase. *Arterioscler. Thromb. Vasc. Biol.* **12:** 1176–1183.
38. Tracey, K. J., and A. Cerami. 1993. Tumor necrosis factor, other cytokines and disease. *Annu. Rev. Cell Biol.* **9:** 317–343.
39. Pickup, J. C., G. D. Chusney, and M. B. Mattock. 2000. The innate immune response and type 2 diabetes: evidence that leptin is associated with a stress-related (acute-phase) reaction. *Clin. Endocrinol. (Oxf.).* **52:** 107–112.
40. Fried, S. K., D. A. Bunkin, and A. S. Greenberg. 1998. Omental and subcutaneous adipose tissues of obese subjects release interleukin-6: depot difference and regulation by glucocorticoid. *J. Clin. Endocrinol. Metab.* **83:** 847–850.
41. Vgontzas, A. N., D. A. Papanicolaou, E. O. Bixler, A. Kales, K. Tyson, and G. P. Chrousos. 1997. Elevation of plasma cytokines in disorders of excessive daytime sleepiness: role of sleep disturbance and obesity. *J. Clin. Endocrinol. Metab.* **82:** 1313–1316.
42. de Metz, J., F. Sprangers, E. Endert, M. T. Ackermans, I. J. ten Berge, H. P. Sauerwein, and J. A. Romijn. 1999. Interferon-gamma has immunomodulatory effects with minor endocrine and metabolic effects in humans. *J. Appl. Physiol.* **86:** 517–522.
43. Straczkowski, M., P. Lewczuk, S. Dzienis-Straczkowska, I. Kowalska, A. Stepien, and I. Kinalska. 2002. Elevated soluble intercellular adhesion molecule-1 levels in obesity: relationship to insulin resistance and tumor necrosis factor-alpha system activity. *Metabolism.* **51:** 75–78.
44. Aitman, T. J., A. M. Glazier, C. A. Wallace, L. D. Cooper, P. J. Norsworthy, F. N. Wahid, K. M. Al-Majail, P. M. Trembling, C. J. Mann, C. C. Shoulders, D. Graf, E. St. Lezin, T. W. Kurtz, V. Kren, M. Pravenec, A. Ibrahimi, N. A. Abumrad, L. W. Stanton, and J. Scott. 1999. Identification of Cd36 (Fat) as an insulin-resistance gene causing defective fatty acid and glucose metabolism in hypertensive rats. *Nat. Genet.* **21:** 76–83.
45. Steppan, C. M., S. T. Bailey, S. Bhat, E. J. Brown, R. R. Banerjee, C. M. Wright, H. R. Patel, R. S. Ahima, and M. A. Lazar. 2001. The hormone resistin links obesity to diabetes. *Nature.* **409:** 307–312.