Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 56 (2007) 656–661
Metabolism
Clinical and Experimental
www.elsevier.com/locate/metabol

Lack of association of *CPT1A* polymorphisms or haplotypes on hepatic lipid content or insulin resistance in Japanese individuals with type 2 diabetes mellitus

Yushi Hirota[a,*], Takeshi Ohara[a], Masako Zenibayashi[a], Shin-ichi Kuno[b,c], Keiko Fukuyama[a], Tetsuya Teranishi[a], Kunichi Kouyama[a], Kazuaki Miyake[a], Eiichi Maeda[c], Masato Kasuga[a]

[a]*Division of Diabetes and Digestive and Kidney Diseases, Department of Clinical Molecular Medicine, Kobe University Graduate School of Medicine, Kobe 650-0017, Japan*
[b]*Department of Clinical Study Management, Translational Research Informatics Center, Foundation for Biomedical Research and Innovation, Kobe 650-0047, Japan*
[c]*Clinical Genome Informatics Center, Kobe University Graduate School of Medicine, Kobe 650-0017, Japan*

Received 26 October 2006; accepted 18 December 2006

Abstract

Accumulation of fat in the liver is associated with insulin resistance and type 2 diabetes mellitus. The carnitine palmitoyltransferase (CPT) enzyme system facilitates the transport of long-chain fatty acids into mitochondria, and the gene for the hepatic isoform of CPT1 (*CPT1A*) is a candidate gene for metabolic disorders such as insulin resistance associated with fatty liver. We have now investigated the contribution of the *CPT1A* locus to hepatic lipid content (HLC), insulin resistance, and susceptibility to type 2 diabetes mellitus. A total of 324 type 2 diabetic patients and 300 nondiabetic individuals were enrolled in the study. Eighty-seven of the type 2 diabetic patients who had not been treated with insulin or lipid-lowering drugs were evaluated by homeostasis model assessment for insulin resistance and were subjected to nuclear magnetic resonance for determination of HLC. A total of 19 single nucleotide polymorphisms (SNPs) were identified at the *CPT1A* locus, and linkage disequilibrium analysis revealed a strong linkage disequilibrium block between SNP8 (intron 5) and SNP17 (intron 14). Neither haplotypes nor SNPs of *CPT1A* were found to be associated either with susceptibility to type 2 diabetes mellitus or with HLC or insulin resistance in type 2 diabetic patients.

1. Introduction

Insulin resistance plays an important role in the development of type 2 diabetes mellitus and is determined by both environmental and genetic factors. Although a genetic contribution to insulin resistance has been established, the underlying susceptibility genes are largely unknown [1].

Fat accumulation in the liver is associated with both insulin resistance and type 2 diabetes mellitus [2-8]. The β-oxidation of long-chain fatty acids in mitochondria is a major source of energy, and the carnitine palmitoyltransferase (CPT) enzyme system facilitates the transport of such fatty acids into the mitochondria [9,10]. Carnitine palmitoyltransferase 1, which is localized within the mitochondrial outer membrane, catalyzes the esterification of acyl-coenzyme A to carnitine [11], which is the rate-limiting step of mitochondrial fatty acid oxidation. Three isoforms of CPT1 that are encoded by distinct genes and exhibit distinct tissue distributions have been identified and have been designated by liver (CPT1A or L-CPT1) [12], muscle (CPT1B or M-CPT1) [13], and brain (CPT1C) [14] isoforms. Consistent with their designations, CPT1B is expressed predominantly in skeletal muscle, heart, and adipose tissue, and CPT1C is expressed in the brain, whereas CPT1A has a more widespread distribution but is most abundant in liver. In the liver, it is therefore CPT1A that plays the major role in fatty acid oxidation. The gene for the hepatic isoform of CPT1 (*CPT1A*) is located in human chromosomal region 11q13.1-q13.5 and consists of 1 noncoding exon and 18 coding exons [15]. Mutations of *CPT1A* are responsible for a rare autosomal recessive

* Corresponding author. Tel.: +81 78 382 5861; fax: +81 78 382 2080.
E-mail address: hirota@med.kobe-u.ac.jp (Y. Hirota).

0026-0495/$ – see front matter © 2007 Published by Elsevier Inc.
doi:10.1016/j.metabol.2006.12.014

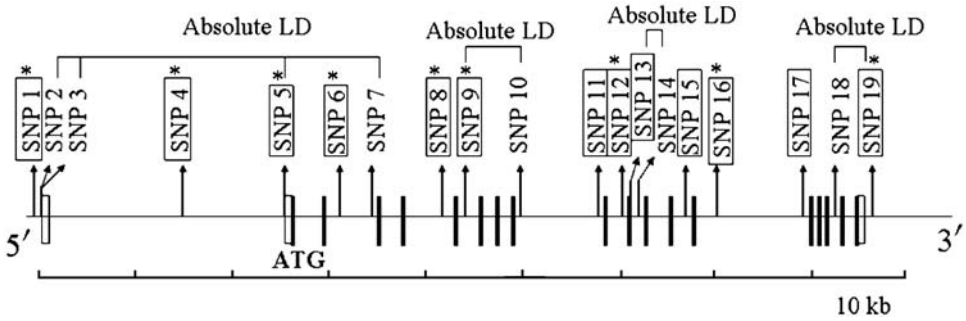

Fig. 1. Single nucleotide polymorphisms of *CPT1A*. Coding and noncoding sequences of exons are represented by closed and open boxes, respectively. Single nucleotide polymorphisms indicated by asterisks and open boxes were used for association and LD analyses, respectively.

disorder, CPT1A deficiency (Online Mendelian Inheritance in Man [OMIM] no. 255120), that has been identified in more than 30 individuals [16-19]. Adults and children with inborn errors in fatty acid oxidation exhibit severe metabolic disturbances including hypoketotic hypoglycemia and fatty liver [20].

CPT1A is a candidate gene for metabolic disorders such as insulin resistance associated with fatty liver [21]. However, as far as we are aware, no genetic association studies have focused on the relation of *CPT1A* to insulin resistance or type 2 diabetes mellitus. We have now investigated whether *CPT1A* might be a determinant of hepatic lipid content (HLC), insulin resistance, or susceptibility to type 2 diabetes mellitus.

2. Materials and methods

2.1. Study population

A total of 324 patients with type 2 diabetes mellitus (176 men and 148 women; age at testing, 62.4 ± 10.7 years; body mass index [BMI], 24.1 ± 3.8 kg/m^2; hemoglobin A_{1c} [HbA_{1c}] level, 8.0% ± 1.9%) and 300 nondiabetic individuals (139 men and 161 women; age at testing, 74.6 ± 8.0 years; BMI, 21.6 ± 3.6 kg/m^2; HbA_{1c} level, 5.0 ± 0.4%) were enrolled in the study. All individuals were Japanese. Diabetes was diagnosed according to the criteria of the American Diabetes Association (1997), and the nondiabetic individuals were selected according to the following criteria: age of older than 60 years, no history of glucose intolerance, HbA_{1c} level of less than 5.6%, and no family history of diabetes mellitus. A total of 87 diabetic patients (51 men, 36 women) who had not been treated with insulin or lipid-lowering drugs were both evaluated by the homeostasis model assessment for insulin resistance (HOMA-IR) and subjected to determination of HLC by nuclear magnetic resonance. The study was performed with written informed consent from all individuals and was approved by the ethics committee of the Kobe University Graduate School of Medicine.

Table 1
Single nucleotide polymorphisms of *CPT1A* examined in the present study

SNP	Position[a] (base pairs)	Location	Major/minor alleles	dbSNP ID[b]	MAF[c]
SNP1	−27841	5′ Flanking	C/G	rs597316	0.25
SNP2	−26461	5′ Flanking	C/G	Novel	0.05
SNP3	−26422	5′ Flanking	C/G	Novel	0.05
SNP4	−12281	Intron 1	G/A	rs879784	0.34
SNP5	−702	Intron 1	A/G	rs2924689	0.05
SNP6	3374	Intron 3	G/C	rs2278906	0.07
SNP7	7767	Intron 3	A/G	rs6591356	0.05
SNP8	14283	Intron 5	G/A	rs3794020	0.32
SNP9	16721	Intron 6	T/C	rs2924675	0.11
SNP10	22189	Intron 9	G/A	Novel	0.11
SNP11	30483	Intron 9	C/T	rs2305507	0.11
SNP12	33423	Intron 10	A/G	rs2305508	0.18
SNP13	33629	Exon 11	C/T	rs2228502	0.14
SNP14	34709	Intron 11	T/G	rs3019576	0.14
SNP15	42019	Intron 13	G/A	Novel	0.05
SNP16	44309	Intron 14	T/C	rs2123869	0.16
SNP17	52712	Intron 14	G/A	rs2924699	0.11
SNP18	55369	Intron 17	C/T	rs3019598	0.27
SNP19	59059	3′ Flanking	G/A	rs2278908	0.27

[a] Position relative to the translation initiation site in the genomic sequence (GenBank accession no. NT_033903).
[b] Reference SNP ID (rs) in SNP database by NCBI: http://www.ncbi.nlm.nih.gov/SNP.
[c] Minor allele frequency determined by the 22 DNA samples by direct sequencing.

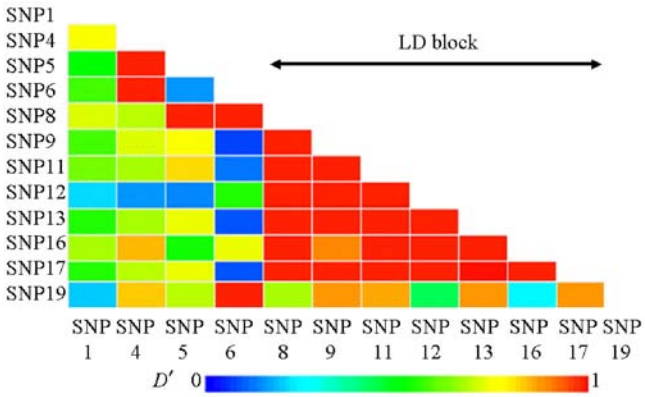

Fig. 2. Pairwise LD analysis for SNPs of *CPT1A*. Pairwise LD between SNPs was evaluated from D' in 86 nondiabetic individuals. A strong LD block was identified between SNP8 (intron 5) and SNP17 (intron 14).

2.2. Clinical assessment

The BMI of each individual was directly measured at the time of collection of blood samples. The fasting plasma glucose (FPG) concentration, fasting plasma immunoreactive insulin (FIRI) concentration, serum lipid concentrations, and HbA_{1c} level were determined by standard laboratory techniques calibrated with uniform standards. Homeostasis model assessment for insulin resistance, calculated as [FPG (mmol/L) × FIRI (μU/mL)]/22.5, was used as an index of insulin resistance [22].

2.3. Determination of HLC

The intracellular lipid content of the liver (HLC) was determined by nuclear magnetic resonance as described previously [23]. In brief, we used the fast spoiled gradient recalled acquisition in the steady-state sequence to obtain in-phase and out-of-phase images of the liver and then calculated HLC as [(intensities of in-phase) − (intensities of out-of-phase)]/[(intensities of in-phase) + (intensities of out-of-phase)].

2.4. Sequencing of CPT1A and genotyping of single nucleotide polymorphisms

Genomic DNA was extracted from blood with a QIAamp DNA Blood Maxi Kit (Qiagen, Valencia, CA). Specific oligonucleotide primer sets were designed on the basis of the *CPT1A* sequence information in GenBank (accession number NT_033903). Regions encompassing all coding exons, exon-intron boundaries, and the 5′ flanking sequence of *CPT1A* were amplified by the polymerase chain reaction (PCR) from genomic DNA of 22 patients with type 2 diabetes mellitus. The conditions and the sequences of primers are available from the authors. Sequencing of PCR products was performed with a Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) and an ABI 3100 DNA automated sequencer (Applied Biosystems). We selected 7 single nucleotide polymorphisms (SNPs) from the public database (rs597316, SNP1; rs879784, SNP4; rs2278906, SNP6; rs3794020, SNP8; rs2924675, SNP9; rs2123869, SNP16; and rs2278908, SNP19) and determined their genotypes by direct sequencing of the PCR products for verification of other genotyping methods. The genotypes of 4 SNPs (rs2924689, SNP5; rs3794020, SNP8; rs2305507, SNP11; and rs2305508, SNP12) were determined by the TaqMan procedure with an ABI Prism 7700 instrument (Applied Biosystems). The genotypes of 9 other SNPs (rs597316, SNP1; rs879784, SNP4; rs2278906, SNP6; rs2924675, SNP9; rs2228502, SNP13; novel SNP, SNP15; rs2123869, SNP16; rs2924699, SNP17; and rs2278908, SNP19) were determined by the intercalator-mediated fluorescence resonance energy transfer probe method (Toyobo Gene Analysis, Tsuruga, Japan). In brief, the reaction was performed in a volume of 25 μL containing 20 ng of genomic DNA, enzyme reaction buffer, 1.5 to 3.5 mmol/L $MgCl_2$, 0.2 mmol/L deoxynucleoside triphosphates, and 1.25 U of rTaq containing anti-Taqplus High (Toyobo, Osaka, Japan). The amplification protocol was performed in a PE 9700 thermocycler (Applied Biosystems) and included an initial denaturation at 95°C for 5 minutes; 40 or 45 cycles of denaturation at 95°C for 30 seconds, annealing at 65°C for 30 seconds, and primer extension at 72°C for 30 seconds; and a final extension at 72°C for 2 minutes. The PCR products were then mixed with 5 μL of a solution containing 40 mmol/L EDTA, 10 pmol of Texas red–labeled probe, and SYBR Green I (Invitrogen, Carlsbad, CA; final concentration, ×25 000) and transferred to an ABI Prism 7700 instrument for measurement of the melting temperature with incubations at 95°C for 30 seconds, 40°C for 1 minute, and temperatures increasing to 80°C over 10 minutes. The fluorescence signal was detected at excitation and emission wavelengths of 485 and 612 nm, respectively. The conditions and primer and probe sequences for genotyping are available from the authors. Genotypes determined by the TaqMan or

Table 2
Common haplotypes of the identified LD block in *CPT1A*

Haplotype	SNP8	SNP9	SNP11	SNP12	SNP13	SNP16	SNP17	Frequency
ht1	G	T	C	A	C	T	G	0.31
ht2	A	T	C	A	C	T	G	0.31
ht3	G	T	C	G	C	T	G	0.25
ht4	G	C	T	A	T	C	A	0.07
ht5	G	T	C	A	C	C	G	0.06

SNP8, SNP9, SNP12, and SNP16 are HT-SNPs for distinguishing these 5 common haplotypes.

Table 3
Association analysis for SNPs of *CPT1A* and type 2 diabetes mellitus

SNP	M/m alleles	Genotype distribution (MM/Mm/mm)		Minor allele frequency		*P*	
		Case	Control	Case	Control	Genotype	Allele
SNP1	C/G	208/105/11	193/90/17	0.20	0.21	.35	.64
SNP4	G/A	87/166/71	68/157/74	0.48	0.51	.44	.22
SNP5	A/G	252/67/4	234/45/4	0.12	0.09	.31	.20
SNP6	G/C	272/44/2	256/43/1	0.07	0.08	.85	.98
SNP8	G/A	150/139/33	135/119/33	0.32	0.32	.85	.88
SNP9	T/C	267/55/2	257/43/0	0.09	0.07	.25	.21
SNP12	A/G	194/113/16	174/92/18	0.22	0.23	.65	.97
SNP16	T/C	245/74/4	238/54/5	0.13	0.11	.32	.29
SNP19	G/A	200/105/19	180/103/13	0.22	0.22	.63	.91

M and m indicate major and minor alleles, respectively.

intercalator-mediated fluorescence resonance energy transfer probe methods were identical to those determined by direct sequencing of PCR products for the 22 DNA samples subjected to this latter analysis.

2.5. Statistical analysis

Averaged data are presented as means ± SD. We assessed association and Hardy-Weinberg equilibrium by the χ^2 test. Linkage disequilibrium (LD) and haplotype analyses were performed with SNPAlyze software version 5.0 Pro (Dynacom, Chiba, Japan). Haplotype estimation was performed by the expectation-maximization algorithm. To measure LD between SNPs, we calculated D' for 86 nondiabetic individuals. Statistical analysis was performed using StatView software version 5.0-J (SAS Institute, Cary, NC). Comparisons among several groups were performed using the Kruskal-Wallis test, and categorical variables were compared using the χ^2 test. A P value of less than .05 was considered statistically significant.

3. Results

We identified a total of 19 SNPs at the *CPT1A* locus (Fig. 1, Table 1). Genotyping of these 19 SNPs in 22 diabetic patients revealed 4 groups of polymorphisms in absolute LD: group 1, SNP2, SNP3, SNP5, and SNP7; group 2, SNP9 and SNP10; group 3, SNP13 and SNP14; group 4, SNP18 and SNP19 (Fig. 1). We selected one SNP from each group and then genotyped these 4 representative SNPs together with the 9 remaining polymorphisms in 86 nondiabetic individuals to identify LD blocks. The genotype frequency for SNP15 was not in Hardy-Weinberg equilibrium ($P < 0.05$), and this SNP was therefore not analyzed further. Linkage disequilibrium analysis identified a strong LD block between SNP8 (intron 5) and SNP17 (intron 14) (Fig. 2). We identified 5 common haplotypes for the 7 SNPs in the LD block as well as 4 haplotype-tagging SNPs (HT-SNPs) to distinguish these 5 common haplotypes and to capture the most haplotype diversity [24] (Table 2). We then expanded genotyping for these 4 HT-SNPs (SNP8, SNP9, SNP12, SNP16) and the 5 SNPs outside of the LD block (SNP1, SNP4, SNP5, SNP6, SNP19) to all 624 samples to perform an association study. No significant associations between these SNPs and type 2 diabetes mellitus were observed (Table 3). Furthermore, haplotype analysis revealed that the frequencies of the haplotypes for the LD block did not differ significantly between cases and controls (data not shown). These 5 haplotypes accounted for 99% of all chromosomes.

We next analyzed the possible effects of haplotype on HLC, HOMA-IR, and serum triglyceride concentration in 87 type 2 diabetic patients. We detected a borderline association for the *ht2* haplotype of the LD block and HOMA-IR ($P = .066$). However, this haplotype was not

Table 4
Clinical profiles of type 2 diabetic patients according to genotype for the *ht2* haplotype of *CPT1A*

Characteristic	−/− (n = 40)	−/*ht2* (n = 37)	*ht2*/*ht2* (n = 10)	*P*
Sex (male/female)	26/14	21/16	4/6	.34[a]
Age (y)	58.2 ± 13.6	60.4 ± 12.5	56.8 ± 10.9	.47
BMI (kg/m^2)	25.0 ± 4.2	25.3 ± 3.7	27.0 ± 6.0	.69
FPG (mmol/L)	7.4 ± 1.9	7.5 ± 2.5	7.5 ± 1.6	.75
FIRI (pmol/L)	46.6 ± 39.7	49.0 ± 31.5	92.3 ± 65.3	.064
HOMA-IR	2.7 ± 2.1	3.0 ± 2.3	5.2 ± 3.5	.066
Total serum cholesterol (mmol/L)	5.40 ± 0.88	5.35 ± 0.89	5.53 ± 1.06	.73
Serum triglyceride (mmol/L)	1.72 ± 0.89	1.72 ± 0.81	1.65 ± 0.63	.97
Serum HDL cholesterol (mmol/L)	1.22 ± 0.37	1.19 ± 0.27	1.18 ± 0.17	.97
HLC (U)	9.1 ± 6.2	9.5 ± 6.7	12.9 ± 8.1	.34

Statistical analysis was performed using the Kruskal-Wallis test, unless specified otherwise. Data are expressed as means ± SD. HDL indicates high density lipoprotein.
[a] χ^2 Test.

associated with HLC (P = .34) or with serum triglyceride concentration (P = .97) (Table 4). Although we measured HOMA-IR in an additional 149 type 2 diabetic patients, we still did not detect a significant association with *ht2* (3.5 ± 5.9, 2.9 ± 2.5, and 4.3 ± 3.8 for −/−, −/+, and +/+, respectively; n = 236, P = .29). We also examined the possible association of individual SNPs inside and outside of the LD block (SNP1, SNP4, SNP5, SNP6, SNP8, SNP9, SNP12, SNP16, SNP19) with HOMA-IR or HLC. No significant differences in these parameters were apparent among genotypes for any of these SNPs (data not shown).

4. Discussion

Our results failed to reveal an association between SNPs or common haplotypes of *CPT1A* and type 2 diabetes mellitus. Furthermore, we found no association between *CPT1A* haplotypes or SNPs and either an index of insulin resistance or HLC in Japanese with type 2 diabetes mellitus.

Although about 30 mutations of *CPT1A* have been described [16-20], as far as we are aware, our results constitute the first association study for *CPT1A* variants and insulin resistance or type 2 diabetes mellitus. We screened all exons by direct sequencing of PCR products from 22 DNA samples. One variant was detected in exon 11 (C>T at position 33 629) that did not result in a change in amino acid (Phe417Phe) and had a minor allele frequency of 13.6%. Screening of 22 DNA samples was sufficient to detect common polymorphisms but not rare ones. Our screening for *CPT1A* variants indicates that reported functional or putatively functional mutations are not common in the Japanese population.

We identified and analyzed 19 SNPs at the *CPT1A* locus, an average marker density of 1 per 4.8 kilobase (kb) covering a total of 87 kb of DNA sequence. To reduce the risk of type II error in association studies with a relatively small sample size, we analyzed LD between SNPs and performed a haplotype association study for an identified LD block. Statistical analysis based on haplotypes is often more efficient than are separate analyses of individual SNPs [25,26]. However, we found no association of either SNPs or haplotypes with type 2 diabetes mellitus.

Association studies with relevant clinical parameters can be a useful approach to clarification of the role of genetic factors in the pathogenesis of type 2 diabetes mellitus or insulin resistance [27]. We previously showed that the −112A>C polymorphism of the uncoupling protein 1 gene (*UCP1*) is a determinant of hepatic lipid accumulation and the development of insulin resistance in type 2 diabetic patients [28]. Stefan and colleagues [29,30] showed that the −514C>T polymorphism of the hepatic lipase gene (*LIPC*) as well as polymorphisms in the gene for adiponectin receptor 1 (*ADIPOR1*) were associated with liver fat content and insulin resistance. These observations reveal the existence of genetic determinants of liver fat content and insulin resistance, and they suggest that such genetic variants may serve as biomarkers for these conditions.

The CPT enzyme system facilitates the transport of long-chain fatty acids into mitochondria. In the liver, excess long-chain fatty acids are converted into triglycerides, a proportion of which is stored in hepatocytes and the remainder is secreted into the plasma as very low-density lipoprotein, giving rise to hypertriglyceridemia [31]. It is possible that differences in CPT1 activity attributable to genetic polymorphisms affect HLC and serum triglyceride concentration. However, our data failed to reveal a significant association of *CPT1A* polymorphisms with these parameters. Furthermore, $Cpt1a^{+/-}$ mice were recently found not to show an increase in liver fat content or in serum triglyceride concentration [32]. The effects of genetic variants of *CPT1A* in humans are likely to be even smaller than that of *Cpt1a* hemizygosity in mice. Our data thus seem consistent with the phenotype of $Cpt1a^{+/-}$ mice.

In conclusion, we found no evidence for a substantial effect of *CPT1A* haplotypes or SNPs either on susceptibility to type 2 diabetes mellitus or on HLC or insulin resistance in type 2 diabetic patients. However, association studies for genetic polymorphisms and clinical parameters such as HLC may provide insight into the pathogenesis of type 2 diabetes mellitus and insulin resistance in humans. Similar studies with a larger sample size and with nondiabetic individuals are needed to clarify the clinical importance of *CPT1A* in humans.

Acknowledgments

This work was supported by the 21st Century COE Program “Center of Excellence for Signal Transduction Disease: Diabetes Mellitus as Model” and by a Grant-in-Aid for Scientific Research on Priority Areas (C), Medical Genome Science, both awarded to MK by the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

We thank T Fukui and T Katsumura for technical assistance.

References

[1] Almind K, Doria A, Kahn CR. Putting the genes for type II diabetes on the map. Nat Med 2001;7:277-9.

[2] Angulo P. Nonalcoholic fatty liver disease. N Engl J Med 2002;346:1221-31.

[3] Maeda K, Ishihara K, Miyake K, et al. Inverse correlation between serum adiponectin concentration and hepatic lipid content in Japanese with type 2 diabetes. Metabolism 2005;54:775-80.

[4] Banerji MA, Buckley MC, Chaiken RL, et al. Liver fat, serum trglycerides and visceral adipose tissue in insulin-sensitive and insulin-resistant black men with NIDDM. Int J Obes Relat Metab Disord 1995;19:846-50.

[5] Seppala-Lindroos A, Vehkavaara S, Hakkinen AM, et al. Fat accumulation in the liver is associated with defects in insulin suppression of glucose production and serum free fatty acids

independent of obesity in normal men. J Clin Endocrinol Metab 2002;87:3023-8.
[6] Marchesini G, Brizi M, Bianchi G, et al. Nonalcoholic fatty liver disease. A feature of metabolic syndrome. Diabetes 2001;50:1844-50.
[7] Kelley DE, McKolanis TM, Hegazi RA, et al. Fatty liver in type 2 diabetes mellitus: relation to regional adiposity, fatty acids, and insulin resistance. Am J Physiol Endocrinol Metab 2003;285:906-16.
[8] Petersen KF, Dufour S, Befroy D, et al. Reversal of nonalcoholic hepatic steatosis, hepatic insulin resistance, and hyperglycemia by moderate weight reduction in patients with type 2 diabetes. Diabetes 2005;54:603-8.
[9] McGarry JD, Mannaerts GP, Foster DW. A possible role for malonyl-CoA in the regulation of hepatic fatty acid oxidation and ketogenesis. J Clin Invest 1977;60:265-70.
[10] McGarry JD, Brown NF. The mitochondrial carnitine palmitoyltransferase system. From concept to molecular analysis. Eur J Biochem 1997;244:1-14.
[11] Fraser F, Corstorphine CG, Zammit VA. Topology of carnitine palmitoyltransferase I in the mitochondrial outer membrane. Biochem J 1997;323:711-8.
[12] Britton CH, Schultz RA, Zhang B, et al. Human liver mitochondrial carnitine palmitoyltransferase I: characterization of its cDNA and chromosomal localization and partial analysis of the gene. Proc Natl Acad Sci U S A 1995;92:1984-8.
[13] Yamazaki N, Shinohara Y, Shima A, et al. Isolation and characterization of cDNA and genomic clones encoding human muscle type carnitine palmitoyltransferase. Biochim Biophys Acta 1996;1307:157-61.
[14] Price N, van der Leij F, Jackson V, et al. A novel brain-expressed protein related to carnitine palmitoyltransferase I. Genomics 2002;80:433-42.
[15] Gobin S, Bonnefont JP, Prip-Buus C, et al. Organization of the human liver carnitine palmitoyltransferase 1 gene (CPT1A) and identification of novel mutations in hypoketotic hypoglycaemia. Hum Genet 2002;111:179-89.
[16] Bennett MJ, Boriack RL, Narayan S, et al. Novel mutations in CPT 1A define molecular heterogeneity of hepatic carnitine palmitoyltransferase I deficiency. Mol Genet Metab 2004;82:59-63.
[17] Ijlst L, Mandel H, Oostheim W, et al. Molecular basis of hepatic carnitine palmitoyltransferase I deficiency. J Clin Invest 1998;102:527-31.
[18] Brown NF, Mullur RS, Subramanian I, et al. Molecular characterization of L-CPT I deficiency in six patients: insights into function of the native enzyme. J Lipid Res 2001;42:1134-42.
[19] Ogawa E, Kanazawa M, Yamamoto S, et al. Expression analysis of two mutations in carnitine palmitoyltransferase IA deficiency. J Hum Genet 2002;47:342-7.
[20] Sim KG, Hammond J, Wilcken B. Strategies for the diagnosis of mitochondrial fatty acid β-oxidation disorders. Clin Chim Acta 2002;323:37-58.
[21] McGarry JD. Banting Lecture 2001. Dysregulation of fatty acid metabolism in the etiology of type 2 diabetes. Diabetes 2002;51:7-18.
[22] Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412-9.
[23] Kawamitsu H, Kaji Y, Ohara T, et al. Feasibility of quantitative intrahepatic lipid imaging applied to the magnetic resonance dual gradient echo sequence. Magn Reson Med Sci 2003;2:47-50.
[24] Johnson GC, Esposito L, Barratt BJ, et al. Haplotype tagging for the identification of common disease genes. Nat Genet 2001;29:233-7.
[25] Zhang K, Calabrese P, Nordborg M, et al. Haplotype block structure and its applications to association studies: power and study designs. Am J Hum Genet 2002;71:1386-94.
[26] Morris RW, Kaplan NL. On the advantage of haplotype analysis in the presence of multiple disease susceptibility alleles. Genet Epidemiol 2002;23:221-33.
[27] Bougneres P. Genetics of obesity and type 2 diabetes: tracking pathogenic traits during the predisease period. Diabetes 2002;51:S295-S303.
[28] Fukuyama K, Ohara T, Hirota Y, et al. Association of the −112A>C polymorphism of the uncoupling protein 1 gene with insulin resistance in Japanese individuals with type 2 diabetes. Biochem Biophys Res Commun 2006;339:1212-6.
[29] Stefan N, Machicao F, Staiger H, et al. Polymorphisms in the gene encoding adiponectin receptor 1 are associated with insulin resistance and high liver fat. Diabetologia 2005;48:2282-91.
[30] Stefan N, Schafer S, Machicao F, et al. Liver fat and insulin resistance are independently associated with the −514C>T polymorphism of the hepatic lipase gene. J Clin Endocrinol Metab 2005;90:4238-43.
[31] Pessayre D, Mansouri A, Fromenty B. Nonalcoholic steatosis and steatohepatitis. V. Mitochondrial dysfunction in steatohepatitis. Am J Physiol Gastrointest Liver Physiol 2002;282:G193–9.
[32] Nyman LR, Cox KB, Hoppel CL, et al. Homozygous carnitine palmitoyltransferase 1a (liver isoform) deficiency is lethal in the mouse. Mol Genet Metab 2005;86:179-87.