Brief Genetics Report

A Novel Missense Substitution (Val1483Ile) in the Fatty Acid Synthase Gene (*FAS*) Is Associated With Percentage of Body Fat and Substrate Oxidation Rates in Nondiabetic Pima Indians

Peter Kovacs, Inge Harper, Robert L. Hanson, Aniello M. Infante, Clifton Bogardus, P. Antonio Tataranni, and Leslie J. Baier

Inhibition of fatty acid synthase (FAS) induces a rapid decline in fat stores in mice, suggesting a role for this enzyme in energy homeostasis. The human FAS gene (*FAS*) maps to chromosome 17q25, a region previously shown to have suggestive linkage to adiposity in a genome-wide linkage scan for genetic determinants of obesity in Pima Indians. To investigate the potential role of *FAS* in the pathophysiology of human obesity, the *FAS* gene was sequenced and 13 single nucleotide polymorphisms (SNPs) were identified. Five representative SNPs were genotyped in 216 full-blooded, nondiabetic Pima Indians for association analyses. A Val1483Ile polymorphism (GTC to ATC; allele frequency of A = 0.10) was associated with percentage of body fat and 24-h substrate oxidation rates measured in a respiratory chamber. Compared with homozygotes for the Val variant, subjects with Ile/x had a lower mean percentage of body fat (30 ± 1 vs. 33 ± 1%, *P* = 0.002; adjusted for age, sex, and family membership) and a lower mean carbohydrate oxidation rate (983 ± 41 vs. 1,094 ± 19 kcal/day, *P* = 0.03), which resulted in a lower mean 24-h respiratory quotient (0.845 ± 0.01 vs. 0.850 ± 0.01 kcal/day, *P* = 0.04; both adjusted for age, sex, family membership, percentage of body fat, and energy balance). Our findings indicate that the Val1483Ile substitution in *FAS* is protective against obesity in Pima Indians, an effect possibly explained by the role of this gene in the regulation of substrate oxidation. *Diabetes* 53:1915–1919, 2004

From the Phoenix Epidemiology and Clinical Research Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Phoenix, Arizona.

Address correspondence and reprint requests to Leslie J. Baier, PhD, Clinical Diabetes and Nutrition Section, NIDDK, NIH, 4212 N. 16th St., Phoenix, AZ 85016. E-mail: lbaier@phx.niddk.nih.gov.

Received for publication 25 November 2003 and accepted in revised form 19 April 2004.

FAS, fatty acid synthase; LOD, logarithm of odds; SNP, single nucleotide polymorphism; UTR, untranslated region.

Obesity is a significant risk factor for many life-threatening diseases, and its global impact on health is enormous (1). Although the etiology of obesity is poorly understood, this disease has both environmental and genetic components. The genetic component is complex and likely has both major gene determinants and minor polygenic determinants. To identify these determinants, we have focused on the relatively genetically and environmentally homogeneous Pima Indian population of southern Arizona. The Pima Indians of Arizona are one of the most obese populations in the world and also have the highest reported prevalence of type 2 diabetes (2). Their diabetes is characterized by obesity, insulin resistance, insulin secretory dysfunction, and increased rates of endogenous glucose production (3,4). To search for obesity susceptibility genes, we previously completed (5,6) a genome-wide linkage scan in Pima Indians. The strongest evidence for linkage with BMI was on chromosome 11q23-24 (logarithm of odds [LOD] = 3.6) (5), whereas the strongest evidence for linkage with percentage of body fat was on chromosome 17q25 (LOD = 1.9) in a multipoint, sibling-based, variance component analysis (6).

The human fatty acid synthase (FAS) gene (*FAS*) is positioned at 135 cM, within 7 cM of the peak of linkage to percentage of body fat on chromosome 17q25 (6). The FAS enzyme is necessary for de novo synthesis of long-chain fatty acids from acetyl-CoA, malonyl-CoA, and NADPH (7). Recent physiologic studies have shown that inhibition of the FAS gene induces a rapid decline in fat stores in mice, suggesting a role for FAS in energy homeostasis (8–12). Based on the chromosomal location and known physiology of FAS, we investigated *FAS* as a candidate gene for determining body weight and percentage of body fat in Pima Indians.

RESEARCH DESIGN AND METHODS

The subjects were participants of our ongoing longitudinal study of the etiology of type 2 diabetes among the Gila River Indian Community in Arizona (3,4). The 216 individuals selected for the association analyses were all nondiabetic subjects who had been admitted to our clinical research ward for

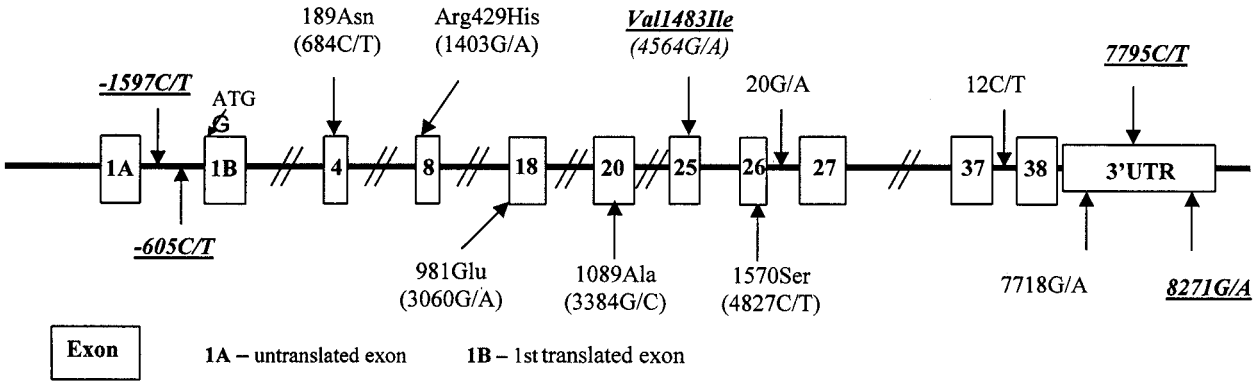

FIG. 1. SNPs detected in the *FAS* gene and their positions. Underlined SNPs in italics were genotyped in a cohort of 216 nondiabetic Pima Indians and analyzed for association with obesity and related phenotypes. Positions of SNPs in exons and the 3′ UTR are based on the cDNA sequence NM_004104.4 (GenBank). Positions of −1597C/T, −605C/T, 20G/A, and 12C/T are based on the genomic contig AC105341 (nucleotides 139340, 138348, 125791, and 122450, respectively).

7–10 days for detailed metabolic studies. All were found to be healthy by medical history, physical examination, and routine laboratory tests and were not taking medications. Oral glucose tolerance was measured after 2–3 days on a weight-maintaining diet of mixed composition. Subjects ingested 75 g of glucose, and blood for plasma glucose and insulin measurements was drawn before ingesting the glucose and at 30, 60, 120, and 180 min thereafter (13). Body composition was estimated by underwater weighing until January 1996 and by dual-energy X-ray absorptiometry (DPX-1; Lunar Radiation, Madison, WI) thereafter. A conversion equation derived from comparative analyses was used to make estimates of body composition equivalent between methods (6). The measurements of energy expenditure and substrate oxidation in the respiratory chamber have been described previously (6). Briefly, volunteers entered the chamber after an overnight fast and remained in the chamber for 23 h. Subjects were fed calories to maintain energy balance according to previously determined equations, and the rate of energy expenditure was measured continuously, calculated for each 15-min interval within the chamber and then extrapolated to 24 h. Carbon dioxide production ($V\mathrm{CO}_2$) and oxygen consumption ($V\mathrm{O}_2$) were calculated for every 15-min interval. The 24-h respiratory quotient was calculated as the ratio of 24-h $V\mathrm{CO}_2$ and 24-h $V\mathrm{O}_2$. Based upon the 24-h respiratory quotient, 24-h metabolic rate, and 24-h urinary nitrogen excretion, the 24-h oxidation rates of fat, carbohydrate, and protein were determined (6). All studies were approved by the Tribal Council and the Institutional Review Board of the National Institutes of Diabetes and Digestive and Kidney Diseases.

Sequencing of the FAS gene. To identify genetic variants, all 43 exons, including intron/exon splicing sites, the 5′ region (1,958 bp upstream of the first translation initiation site), and the 3′ untranslated region (UTR) were sequenced in DNA samples from 32 full-blooded, non–first degree related Pima Indians. Sequencing was performed using the Big Dye Terminator (Applied Biosystems, Foster City, CA) on an automated DNA capillary sequencer (ABI Prism 3700; Applied Biosystems). Sequence information for all oligonucleotide primers used for variant screening is available upon request.

Genotyping of FAS SNPs. Genotyping of selected SNPs in a cohort of nondiabetic full-blooded Pima Indians (n = 216) representing a subset of individuals from the sibling-based linkage analysis, which is described elsewhere (5), was done using the TaqMan assay (Applied Biosystems) for the −605C/T variant and by direct sequencing (as described above) for the −1597C/T, Val1483Ile, 7795C/T, and 8271G/A variants. The TaqMan genotyping reaction was amplified on a GeneAmp PCR system 9700 (50°C for 2 min, 95°C for 10 min, 95°C for 15 s, and 62°C for 1 min, for 38 cycles), and fluorescence was detected on an ABI Prism 7700 sequence detector (Applied Biosystems).

Statistical analyses. Statistical analyses were performed using the statistical analysis system of the SAS Institute (Cary, NC). For continuous variables, the general estimating equation procedure was used to adjust for the covariates, including family membership, because some subjects were siblings. Plasma insulin concentrations were log transformed before analyses to approximate a normal distribution.

RESULTS

Genetic variation in the FAS gene. The *FAS* was sequenced in 32 Pima Indian DNA samples to detect variation, and 13 SNPs were identified (Fig. 1). Two SNPs were in the 5′ region, a −1597C/T (allele frequency of the T allele = 0.16) and a −605C/T (T = 0.23). Six SNPs were found in the exons, where four predicted silent substitutions and two predicted missense substitutions. SNPs predicting silent substitutions were in exon 4 (684C/T→Asn189; T = 0.06), exon 18 (3060G/A→981Glu; A = 0.02), exon 20 (3384G/C→Ala1089; C = 0.19), and exon 26 (4827C/T→1570Ser; T = 0.02). SNPs predicting missense substitutions were in exon 8 (1403G/A→Arg429His; A = 0.02) and exon 25 (4564G/A→Val1483Ile; A = 0.10). Two SNPs were in introns: intron 26 (20G/A; A = 0.06) and intron 37 (12C/T; T = 0.16; rs4246445 in the National Center for Biotechnology Information SNP Database) and three SNPs were in the 3′UTR (7718G/A, A = 0.02; 7795C/T, T = 0.16; and 8271G/A, A = 0.22).

Based on the genotypic concordance, several of these SNPs were in strong linkage disequilibrium. Among the more common variants (minor allele frequency ≥0.10), the 12C/T variant in intron 37 was in 100% linkage disequilibrium with 7795C/T in the 3′ UTR. Similarly, the −605C/T variant in the promoter was in nearly complete linkage disequilibrium with the 3384G/C variant (Ala1089). The remaining three common SNPs appeared to be genotypically unique. Therefore, five representative common SNPs were selected (−1597C/T, −605C/T, Val1483Ile, 7795C/T, and 8271G/A) for additional genotyping for association analyses. The results of a Hardy-Weinberg equilibrium test were as follows: −1597C/T (P = 0.77), −605C/T (P = 0.39), Val1483Ile (P = 0.02), 7795C/T (P = 0.02), and 8271G/A (P = 0.53).

Association studies. The Val1483Ile polymorphism was associated with percentage of body fat, 24-h carbohydrate oxidation rates, and 24-h respiratory quotient as measured in a respiratory chamber. Compared with Val homozygotes, subjects with Ile/x had a lower mean percentage of body fat (30 ± 1 vs. 33 ± 1%, P = 0.002; adjusted for age, sex, and family membership) and a lower mean 24-h carbohydrate oxidation rate (983 ± 41 vs. 1,094 ± 19 kcal/day, P = 0.03), which resulted in a lower mean 24-h respiratory quotient (0.845 ± 0.01 vs. 0.850 ± 0.01 kcal/day, P = 0.04; both adjusted for age, sex, family membership, percentage of body fat, and energy balance) (Table 1).

The SNP −1597C/T was associated with 24-h metabolic rate ($P < 0.05$ after adjusting for age, sex, family member-

TABLE 1
Metabolic characteristics of nondiabetic Pima Indians grouped by Val1483Ile genotypes

Genotype	Val/Val	Val/Ile + Ile/Ile	*P*
Oral glucose tolerance test			
n	174	40	
Age (years)	28 ± 1	26 ± 1	—
Percentage of body fat	33 ± 1	30 ± 1	**0.002**
Fasting plasma glucose (mg/dl)	92 ± 1	92 ± 1	0.54
2-h plasma glucose (mg/dl)	129 ± 2	119 ± 5	0.39
Fasting plasma insulin (μU/ml)	44 ± 2	37 ± 4	0.16
2-h plasma insulin (μU/ml)	224 ± 13	165 ± 23	0.17
Respiratory chamber			
n	142	32	
24-h respiratory quotient (kcal/day)	0.850 ± 0.01	0.845 ± 0.01	**0.04**
24-h metabolic rate (kcal/day)	2,418 ± 33	2,229 ± 49	0.24
Carbohydrate oxidation (kcal/day)	1,094 ± 19	983 ± 41	**0.03**
Lipid oxidation (kcal/day)	984 ± 26	938 ± 45	0.16

Data are means ± SE. *P* values were calculated after adjusting for age, sex, and family membership for the percentage of body fat variable and for age, sex, family membership, and percentage of body fat for the 2-h plasma glucose and 2-h plasma insulin variables. *P* values for the 24-h respiratory quotient and carbohydrate and lipid oxidation variables were calculated after adjusting for age, sex, family membership, percentage of body fat, and energy balance. *P* values for 24-h metabolic rate were calculated after adjusting for age, sex, family membership, fat-free mass, and fat mass. Due to the low frequency of the rare allele, for statistical analysis the Ile homozygotes were combined with the Val/Ile heterozygotes; therefore, only a dominant effect on risk has been tested for the rare allele. *P* values <0.05 were considered significant and are shown in boldface.

ship, fat mass, and fat-free mass) (Table 2). The SNP −605C/T was modestly associated with fasting plasma glucose ($P < 0.05$), but none of the other variants were associated with any measure of plasma glucose or insulin concentrations that would be predictive of type 2 diabetes (Tables 1 and 2).

In addition, among the five different SNPs genotyped, we identified five common haplotypes (frequency >0.05) that accounted for 88% of the observed haplotypes. These common haplotypes could be defined by four of the SNPs ([−1597C/T]-[−605C/T]-[7795C/T]-[8271G/A]). Each of the common haplotypes was analyzed for its association with metabolic characteristics. To accomplish this, a modification of the zero-recombination haplotyping approach was used (14–16). However, analyses of these haplotypes did not reveal any strong associations beyond those expected from analysis of individual SNPs.

DISCUSSION

FAS was analyzed as a candidate gene for human obesity, and a novel SNP predicting a Val1483Ile substitution was identified that was associated with percentage of body fat and substrate oxidation rates in nondiabetic Pima Indians. In addition, the Val1483Ile variant appears to contribute to the linkage at 17q25, but by itself does not explain all of the linkage at this position, as a Haseman-Elston test for linkage following adjustment for this SNP drops the LOD score from 1.9 to 1.5. Although no reports on genetic variation in human *FAS* have been previously published, there are a number of recent studies from rodents indicating that FAS may be involved in obesity through regulation of feeding behavior. Treatment of mice with FAS inhibitors (cerulenin, C75) resulted in reduced food intake and substantial body weight loss (8). The FAS inhibitor, C75, acts both centrally to reduce food intake and peripherally to increase fatty acid oxidation, leading to rapid and profound weight loss, loss of adipose mass, and resolution of fatty liver in diet-induced obese mice (12). If the Val/Ile substitution functions to reduce the activity of FAS, it might be predicted to be associated with decreased fat in humans. The Val1483Ile variant is positioned within the interdomain region of the *FAS*. Although the interdomain region has no catalytic activity itself, it appears to have an important role in dimer formation. Both human and animal FAS proteins are dimers, composed of two identical multifunctional subunits arranged in an antiparallel configuration, which generates two active centers for fatty acid synthesis separated by the interdomain regions. Therefore, alteration of the interdomain region could affect dimer formation and consequently alter the configuration necessary for catalytically active FAS (17–20). However, further studies are needed to determine whether the Val/Ile substitution indeed structurally alters the interdomain, which in turn reduces FAS activity.

The physiologic mechanism by which the Val1483Ile substitution results in the phenotypic and metabolic changes observed in Pima Indians also requires further study. Our data indicate that the higher percentage of body fat in subjects with the Val/Val genotype results from their higher carbohydrate oxidation rates. As indicated by their higher 24-h respiratory quotient, these Val/Val subjects have an increased ratio of carbohydrate to lipid oxidation rates, i.e., they preferentially burn carbohydrates over lipids, which results in an increased body lipid mass. In mice, inhibition of FAS by C75 leads to profound weight loss via an increase in peripheral fatty acid oxidation, but this effect is achieved by a C75-specific pharmacological stimulation of carnitine palmityltransferase-1, which controls fatty acid entry into the mitochondria for oxidation (12). It is unlikely that the Val1483Ile substitution affects the substrate oxidation rates via a similar mechanism. However, FAS inhibitors also act centrally through inhibition of the orexigenic system of neuropeptide Y and agouti-related protein, leading to a reduced food intake and weight loss in rodents. Therefore, future studies could examine possible relationships between the Val1483Ile

TABLE 2
Metabolic characteristics of nondiabetic Pima Indians grouped by genotypes in representative *FAS* variants

	−1597C/T		−605C/T			7795C/T		8271G/A	
Genotype	C/C	C/T + T/T	C/C	C/T	T/T	C/C	C/T + T/T	G/G	G/A + A/A
Oral glucose tolerance test									
n	167	43	101	84	20	137	65	161	49
Age (years)	27 ± 1	27 ± 1	28 ± 1	26 ± 1	27 ± 1	27 ± 1	28 ± 1	28 ± 1	26 ± 1
Percentage of body fat	33 ± 1	31 ± 1	33 ± 1	32 ± 1	33 ± 2	32 ± 1	33 ± 1	33 ± 1	30 ± 1
Fasting plasma glucose (mg/dl)	92 ± 1	91 ± 1	92 ± 1	92 ± 1	89 ± 2*	92 ± 1	92 ± 1	92 ± 1	92 ± 1
2-h plasma glucose (mg/dl)	129 ± 2	119 ± 5	128 ± 3	123 ± 3	123 ± 8	126 ± 3	128 ± 4	129 ± 3	118 ± 4
Fasting plasma insulin (μU/ml)	44 ± 2	39 ± 3	43 ± 2	43 ± 3	42 ± 5	43 ± 2	40 ± 3	43 ± 2	42 ± 4
2-h plasma insulin (μU/ml)	219 ± 14	200 ± 22	211 ± 15	210 ± 19	219 ± 52	204 ± 13	220 ± 26	225 ± 14	173 ± 21
Respiratory chamber									
n	135	38	89	66	20	118	54	132	43
24-h respiratory quotient (kcal/day)	0.848 ± 0.01	0.852 ± 0.01	0.850 ± 0.01	0.848 ± 0.01	0.848 ± 0.01	0.848 ± 0.01	0.851 ± 0.01	0.850 ± 0.01	0.847 ± 0.01
24-h metabolic rate (kcal/day)	2,362 ± 33	2,402 ± 59†	2,374 ± 46	2,363 ± 39	2,410 ± 92	2,365 ± 37	2,396 ± 48	2,388 ± 34	2,388 ± 34
Carbohydrate oxidation (kcal/day)	1,061 ± 19	1,097 ± 36	1,069 ± 23	1,064 ± 30	1,082 ± 53	1,058 ± 22	1,105 ± 29	1,084 ± 20	1,084 ± 20
Lipid oxidation (kcal/day)	979 ± 25	938 ± 51	972 ± 34	967 ± 30	980 ± 74	973 ± 28	954 ± 37	970 ± 26	970 ± 26

Data are means ± SE. Legend is the same as in Table 1. Due to the low frequency of the rare alleles for the −1597C/T, 7795C/T, and 8271G/A variants, for statistical analysis the homozygotes for each rare allele were combined with the heterozygotes; therefore, only a dominant effect on risk has been tested for the rare alleles. Differences in metabolic characteristics between genotypes were assessed using linear regression models. In these models, percentage of body fat, fasting and 2-h plasma glucose, plasma insulin, 24-h respiratory quotient, 24-h metabolic rate, carbohydrate oxidation, and lipid oxidation were the dependent variables. To account for potential nonindependence in the dependent variable introduced from analysis of family members (i.e., siblings), the regression models were fit using generalized estimating equations (21). These models allow for a common correlation among siblings, but do not conduct within-family association analyses necessary to control confounding by population stratification. Within-family association requires at least two individuals per sibship and, given the limited number of informative sibships in the present study, no such analyses were conducted. *$P < 0.05$, †$P < 0.01$.

variant and food intake in humans, which could partially explain the reduced body fat seen in carriers of the 1483Ile variant. It has also been reported (9) that in lean individuals, FAS inhibitors reduce body weight independent of food intake or hypothalamic neuropeptide Y, suggesting that other uncharacterized neuropeptide pathways may mediate the effects of FAS inhibitors on metabolic rate. Therefore, changes in FAS activity could potentially affect substrate oxidation rates, which would result in the decrease in body fat observed in the Pima Indians. It is also noteworthy that FAS inhibitors are more potent in obese compared with lean mice, suggesting that FAS inhibition may be enhanced by the effects of factors such as insulin, glucose, and fatty acids, which are elevated in obese individuals (9). Therefore the effect of FAS inhibition may be more marked in Pima Indians, an extremely obese population, compared with leaner populations.

Based on our data, we suggest that the human *FAS* may have a role in the regulation of body weight in Pima Indians. Although these findings support previous studies in rodents, replication studies in humans and functional studies with the Val1483Ile substitution are necessary to confirm these findings and define the relative importance of this novel substitution in the pathogenesis of human obesity.

REFERENCES

1. Must A, Spadano J, Coakley EH, Field AE, Colditz G, Dietz WH: The disease burden associated with overweight and obesity. *JAMA* 282:1523–1529, 1999
2. Knowler WC, Bennett PH, Hamman RF, Miller M: Diabetes incidence and prevalence in Pima Indians: a 19-fold greater incidence than in Rochester, Minnesota. *Am J Epidemiol* 108:497–505, 1978
3. Bogardus C, Lillioja S, Howard BV, Reaven G, Mott D: Relationships between insulin secretion, insulin action, and fasting plasma glucose concentration in non-diabetic and non-insulin-dependent diabetic subjects. *J Clin Invest* 74:1238–1246, 1984
4. Lillioja S, Mott DM, Spraul M, Ferraro R, Foley JE, Ravussin E, Knowler WC, Bennett PH, Bogardus C: Insulin resistance and insulin secretory dysfunction as precursors of non-insulin-dependent diabetes mellitus: prospective studies of Pima Indians. *N Engl J Med* 329:1988–1992, 1993
5. Hanson RL, Ehm MG, Pettitt DJ, Prochazka M, Thompson DB, Timberlake D, Foroud T, Kobes S, Baier L, Burns D, Almasy L, Blangero J, Garvey WT, Bennett PH, Bogardus C, Knowler WC: An autosomal genomic scan for loci linked to type 2 diabetes mellitus and body mass index in Pima Indians. *Am J Hum Genet* 63:1130–1138, 1998
6. Norman RA, Tataranni PA, Pratley R, Thompson DB, Hanson RL, Prochazka M, Baier L, Ehm MG, Sakul H, Foroud T, Garvey WT, Burns D, Knowler WC, Bennett PH, Bogardus C, Ravussin E: Autosomal genomic scan for loci linked to obesity and energy metabolism in Pima Indians. *Am J Hum Genet* 62:659–668, 1998
7. Semenkovich CF: Regulation of fatty acid synthase (FAS). *Prog Lipid Res* 36:43–53, 1997
8. Loftus TM, Jaworsky DE, Frehywot GL, Townsend CA, Ronnett GV, Lane MD, Kuhajda FP: Reduced food intake and body weight in mice treated with fatty acid synthase inhibitors. *Science* 288:2379–2381, 2000
9. Mobbs CV, Makimura H: Block the FAS, lose the fat. *Nat Med* 8:335–336, 2002
10. Shimokawa T, Kumar MV, Lane MD: Effect of a fatty acid synthase inhibitor on food intake and expression of hypothalamic neuropeptides. *Proc Natl Acad Sci U S A* 99:66–71, 2002
11. Kumar MV, Shimokawa T, Nagy TR, Lane MD: Differential effects of a centrally acting fatty acid synthase inhibitor in lean and obese mice. *Proc Natl Acad Sci U S A* 99:1921–1925, 2002
12. Thupari JN, Landree LE, Ronnett GV, Kuhajda FP: C75 increases peripheral energy utilization and fatty acid oxidation in diet-induced obesity. *Proc Natl Acad Sci U S A* 99:9498–9502, 2002
13. Lillioja S, Mott DM, Zawadzki JK, Young AA, Abbott WG, Knowler WC, Bennett PH, Moll P, Bogardus C: In vivo insulin action is familial characteristic in nondiabetic Pima Indians. *Diabetes* 36:1329–1335, 1987
14. O'Connell JR: Zero-recombinant haplotyping: applications to fine-mapping using SNPs. *Genet Epidemiol* 19 (Suppl. 1):S64–S70, 2000
15. Lathrop GM, Lalouel JM: Easy calculations of lod scores and genetic risks on small computers. *Am J Hum Genet* 36:460–465, 1984
16. Wolford JK, Gruber JD, Ossowski VM, Vozarova B, Tataranni PA, Bogardus C, Hanson RL: A C-reactive protein promoter polymorphism is associated with type 2 diabetes mellitus in Pima Indians. *Mol Genet Metab* 78:136–144, 2003
17. Chirala SS, Jayakumar A, Gu ZW, Wakil SJ: Human fatty acid synthase: role of interdomain in the formation of catalytically active synthase dimer. *Proc Natl Acad Sci U S A* 98:3104–3108, 2001
18. Jayakumar A, Tai MH, Huang WY, al-Feel W, Hsu M, Abu-Elheiga L, Chirala SS, Wakil SJ: Human fatty acid synthase: properties and molecular cloning. *Proc Natl Acad Sci U S A* 92:8695–8699, 1995
19. Jayakumar A, Huang WY, Raetz B, Chirala SS, Wakil SJ: Cloning and expression of the multifunctional human fatty acid synthase and its subdomains in Escherichia coli. *Proc Natl Acad Sci U S A* 93:14509–14514, 1996
20. Jayakumar A, Chirala SS, Wakil SJ: Human fatty acid synthase: assembling recombinant halves of the fatty acid synthase subunit protein reconstitutes enzyme activity. *Proc Natl Acad Sci U S A* 94:12326–12330, 1997
21. Liang KY, Zeger SL: Longitudinal data analysis using generalized linear models. *Biometrika* 73:13–22, 1986