Variation in the Gene for Muscle-Specific AMP Deaminase Is Associated With Insulin Clearance, a Highly Heritable Trait

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The rising prevalence of the insulin resistance syndrome in our society necessitates a better understanding of the genetic determinants of all aspects of insulin action and metabolism. We evaluated the heritability of insulin sensitivity and the metabolic clearance rate of insulin (MCRI) as quantified by the euglycemic-hyperinsulinemic clamp in 403 Mexican Americans. We tested the candidate gene AMP deaminase 1 (*AMPD1*) for association with insulin-related traits because it codes for an enzyme that has the potential to influence multiple aspects of insulin pharmacodynamics. By converting AMP to inosine monophosphate, AMPD1 plays a major role in regulating cellular AMP levels; AMP activates AMP kinase, an enzyme that modulates cellular energy and insulin action. We determined that nine *AMPD1* single nucleotide polymorphisms (SNPs) defined two haplotype blocks. Insulin clearance was found to have a higher heritability ($h^2 = 0.58$) than fasting insulin ($h^2 = 0.38$) or insulin sensitivity ($h^2 = 0.44$). The MCRI was associated with *AMPD1* SNPs and haplotypes. Insulin clearance is a highly heritable trait, and specific haplotypes within the *AMPD1* gene, which encodes a skeletal muscle–specific protein, are associated with variation in insulin clearance. We postulated that the processes of insulin action and insulin clearance in skeletal muscle are highly regulated and that AMPD1 function may play an important role in these phenomena. *Diabetes* 54: 1222–1227, 2005

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Received for publication 19 October 2004 and accepted in revised form 10 January 2005.

AMPK, AMP-activated protein kinase; LD, linkage disequilibrium; MCRI, metabolic clearance rate of insulin; SNP, single nucleotide polymorphism; TDT, transmission disequilibrium test.

The insulin resistance syndrome (also called the metabolic syndrome) is a clustering of factors associated with an increased risk of coronary artery disease (1). In the U.S., >20% of adults are affected by it (2). Mexican Americans have a high prevalence of hyperinsulinemia and insulin resistance as well as the highest age-specific prevalence of the insulin resistance syndrome (2–4). Thus, by studying a large family-based sample of Mexican-American subjects, we sought to elucidate the genetic determinants of insulin metabolism and action.

In pursuit of this goal, we evaluated insulin phenotypes using the euglycemic-hyperinsulinemic clamp study. Assessment of the glucose infusion rate (M) during the euglycemic-hyperinsulinemic clamp is regarded as the most direct physiological measurement of insulin sensitivity (5,6). The clamp also allows calculation of the metabolic clearance rate of insulin (MCRI). In this family-based study, we assessed the variation of these traits within families and observed for the first time a high heritability for MCRI. To begin to identify specific genes that mediate heritability of insulin-related phenotypes, we selected a candidate gene, AMP deaminase 1 (*AMPD1*), which has been shown to influence skeletal muscle adenine nucleotide levels (7) and which in turn could have effects on other enzymes, such as AMP-activated protein kinase (AMPK), that are known to influence insulin action (8). We show herein that polymorphisms in *AMPD1* are associated with variation in the MCRI.

RESEARCH DESIGN AND METHODS

The University of California at Los Angeles/Cedars-Sinai Mexican-American Coronary Artery Disease Project enrolls families ascertained through a proband with coronary artery disease, as determined by evidence of myocardial infarction on an electrocardiogram or in a hospital record, evidence of atherosclerosis on a coronary angiography, or a history of coronary artery bypass graft or angioplasty (9,10). DNA is obtained from all available family members, and the adult offspring (age 18 years or older) of the proband and the spouses of those offspring are also asked to undergo a series of tests to characterize their metabolic and cardiovascular phenotype. In the present study, 832 subjects from 164 families were genotyped; of these, 403 adult offspring and offspring spouses from 99 families underwent the euglycemic-hyperinsulinemic clamp.

All studies were approved by the Human Subjects Protection Institutional Review Boards at the University of California at Los Angeles and Cedars-Sinai Medical Center. All subjects gave informed consent before participating.

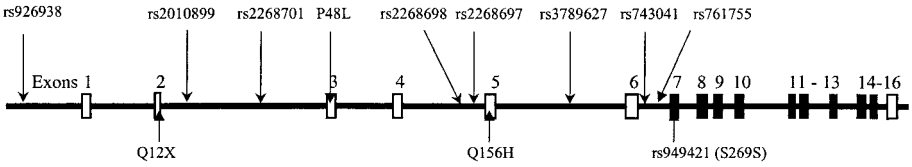

FIG. 1. *AMPD1* gene organization and location of SNPs. The *AMPD1* gene (chromosome 1p13) spans ~22 kb in genomic length. The nine genotyped SNPs that were polymorphic in our population are listed on the top, and the three SNPs not genotyped are listed on the bottom. The width of the exons is slightly exaggerated for clarity. □, exons specific to *AMPD1*; ■, conserved exons shared with *AMPD2* and *AMPD3*.

Genotyping. We genotyped 12 single nucleotide polymorphisms (SNPs) across the *AMPD1* gene (Fig. 1). We selected six variants (rs926938, rs2010899, rs2268698, rs2269697, rs743041, and rs761755) based on the finding that they were commonly shared across different population groups (11). We also genotyped missense variants known to be associated with altered AMPD1 function (Q12X, P48L, and Q156H) (12,13). The remaining variants were selected from the National Center for Biotechnology Information SNP database (www.ncbi.nlm.nih.gov/SNP/). Q12X was incompatible with our genotyping assay, and two variants (Q156H and S269S) were not polymorphic in our Mexican-American population and therefore were not considered further. The nine remaining SNPs were successfully genotyped in 832 subjects from 164 families. For each SNP, 1 represents the major allele and 2 represents the minor allele. This large-scale genotyping was performed using the 5′-exonuclease (Taqman MGB) assay, as previously described (9,14). PCR primer and oligonucleotide probe sequences are listed in Table 1.

Phenotyping. In all, 403 genotyped adult offspring and their spouses underwent a 3-day phenotyping protocol that included indexes of insulin resistance and clearance determined by a euglycemic clamp study on the 3rd day.

During the euglycemic-hyperinsulinemic clamp (5), a priming dose of human insulin (Novolin; Novo Nordisk, Clayton, NC) was given, followed by infusion for 120 min at a constant rate (60 $mU \cdot m^{-2} \cdot min^{-1}$), with the goal of achieving a plasma insulin concentration of 100 μIU/ml or greater. Blood was sampled every 5 min, and the rate of 20% dextrose coinfused was adjusted to maintain plasma glucose concentrations at 95–100 mg/dl. The glucose infusion rate (M; given in milligrams per kilogram per minute) over the last 30 min of steady-state insulin and glucose concentrations reflects glucose uptake by all body tissues (primarily insulin-mediated glucose uptake in muscle) and is therefore a direct physiological measurement of tissue insulin sensitivity (5). Often, an insulin sensitivity index (S_i) is calculated as M/I, where I is the steady-state insulin level. In this study, to clearly distinguish between insulin sensitivity and insulin clearance, we relied on M as the insulin sensitivity measure because the calculations of S_i and insulin clearance both use steady-state insulin in the denominator.

The plasma insulin levels during the steady state of the clamp study are a direct reflection of the MCRI. The MCRI (milliliters per meter squared per minute) was calculated as the insulin infusion rate divided by the final steady-state plasma insulin level of the euglycemic clamp. This formula was chosen because the hyperinsulinemic infusion is known to suppress endogenous insulin secretion; furthermore, in vivo tissue clearance mechanisms do not distinguish between endogenously secreted insulin molecules and infused insulin. Because this measurement of MCRI assesses both, it provides the most accurately available measure of overall insulin clearance in this data set.

Data analysis. Log-transformed trait values (BMI, fasting insulin, and MCRI) or square root−transformed values (M) were used to reduce skewness for all statistical analyses. Unpaired, two-sided *t* tests were used to compare trait values between men and women.

The pairwise relation of age, BMI, fasting insulin, M, and MCRI were individually assessed using simple regression. *P* values were derived using generalized estimating equations to account for familial relationships (15). Generalized estimating equations were used to assess the joint effects of age, BMI, sex, M, and MCRI on fasting insulin, adjusting for familial relationships.

Heritability estimates were obtained using the SOLAR (Sequential Oligogenic Linkage Analysis Routines) program (16) to implement a variance components approach. The total phenotypic variance in a trait (σ^2_P) was partitioned into the variance due to the additive effects of genes (σ^2_G) and environmental effects (σ^2_E). The genetic effect was assumed to be independent and normally distributed with zero mean and variance of σ^2_G. Heritability (h^2) of a trait was calculated by the ratio of the genetic variance (σ^2_G) divided by the total phenotypic variation.

The program Haploview was used to determine haplotypes as well as to delineate haplotype blocks (17). Haploview constructs haplotypes by using an accelerated expectation maximization algorithm, similar to the partition/ligation method (18), which creates highly accurate population frequency estimates of the phased haplotypes based on the maximum likelihood derived from the unphased input genotypes. Haploview was used to calculate linkage disequilibrium (LD; the D′ statistic) between each pairwise combination of all nine SNPs used in the haplotype block determination. To determine haplotype blocks, Haploview searches for regions of strong LD ($D' > 0.8$) running from one marker to another, wherein the first and last markers in a block are in strong LD with all intermediate markers.

Association was evaluated by quantitative transmission disequilibrium testing for individual polymorphisms and haplotypes using the QTDT program (19). The transmission disequilibrium test (TDT) was first developed for dichotomous traits in which alleles transmitted and not transmitted from parents to affected offspring are compared to determine whether one allele is associated with the disease in question (20). The TDT was later extended to quantitative traits (21). Abecasis et al. (19) developed a general approach for scoring allelic transmission that accommodates families of any size and uses

TABLE 1
Primers and probe sequences used in the 5′-exonuclease assay

Variant	PCR primers (5′ to 3′)	Taqman MGB probes (5′ to 3′)
rs926938	TTGTAAGAAAGGTAGGGAAA	FAM-AGTTGCCTACCCAgAG
	ATCTCATTCCAATTGTGTAAATAC	VIC-AGTTGCCTACCCAaAG
rs2010899	AGCCTCCCAAAGTGCTATGATTAC	FAM-AAACCTATTTgAGATAAA
	CAAAAGGACAAGTCTAATTTGAGATTGA	VIC-AAACCTATTTtAGATAAAAC
rs2268701	AATCAATTCCTGTCATCTCCAATG	FAM-CATTTCATCATaTACTAAGTC
	GGTTAGCTTTGGACTATGAATGAGATAA	VIC-CATTTCATCATgTACTAAGT
P48L	TCCCCCTTTGATGTGGATGA	FAM-ATCTGTCcGATTTC
	GGAATATGTGTGCTTGCATCTCA	VIC-TCTGTCtGATTTCTC
rs2268698	CAACCAGGAGTTGGGAATGG	FAM-TTCCAGCAGcTCTAA
	AAAACTTGAGAGATTCTAGAGGAAAGCTT	VIC-TTCCAGCAGgTCTAA
rs2268697	CGACTAACTTCTCACAGAATGATGTTG	FAM-CAGCTACaATGGATC
	CCCATGCACGGCTTTCTT	VIC-CAGCTACgATGGATC
rs3789627	GTGGTTTTCCCATCCTCTATAATATAGAC	FAM-TTAGTTTGTCTTgTCTTTA
	AATTATTATTGAAGCGTAACTTAGGAGAAAT	VIC-TTTAGTTTGTCTTaTCTTTAT
rs743041	CCCTCATTCTTATGTCCAACATTA	FAM-ATCCCACTGAgAAGTA
	ATCAGGACCTTTATCATTCATAGGA	VIC-ATCCCACTGAaAAGTA
rs761755	CAGTGGGATTAACTGCAGAGTAAACT	FAM-AGGTTTGTTCCAAcAAT
	TCAGGACCTTTATCATTCATAGGAAA	VIC-AGGTTTGTTCCAAtAAT

MGB, minor groove binder.

TABLE 2
Clinical characteristics of subjects

	Women	Men
n	235	168
Age (years)	34.9 ± 8.8 (18–58)	34.3 ± 9.3 (19–60)
BMI (kg/m^2)	29.0 ± 5.4 (18.1–54.8)	28.7 ± 4.7 (17.8–45.4)
Fasting insulin (μIU/ml)	13.8 ± 7.4 (3.1–63.1)	15.0 ± 9.1 (2.8–50.1)
Glucose infusion rate (mg · kg^{-1} · min^{-1})	5.4 ± 2.7 (0.2–16.2)	5.8 ± 2.9 (1.0–14.2)
Metabolic clearance rate of insulin (ml · m^{-2} · min^{-1})	536.3 ± 289.5 (174.0–4,285.5)	530.2 ± 256.5 (209.1–2,858.4)

Data are means ± SD (range).

all available genotypic information. Family data allow the construction of an expected genotype for every nonfounder, and orthogonal deviates from this expectation are a measure of allelic transmission. The QTDT program implements this general TDT using the orthogonal model of Abecasis et al. (22). In our study, age, sex, and BMI were specified as covariates. Environmental variance, polygenic variance, and additive major locus were specified in the variance model. The within-family component of association was evaluated to eliminate any effects of population stratification.

RESULTS

The clinical characteristics of the 403 subjects (168 men, 235 women) who underwent clamp assessment of insulin resistance are shown in Table 2. There were no significant differences between the men and women in anthropometric or insulin-related traits.

Both M and MCRI were negatively correlated with the fasting insulin concentration ($P < 0.0001$ for both comparisons) (Table 3). There was a weak correlation between MCRI and M ($r = 0.085$, $P = 0.032$). BMI was highly correlated with M ($r = -0.58$, $P < 0.0001$) but only weakly correlated with MCRI ($r = -0.11$, $P = 0.016$).

Age, sex, BMI, M, and MCRI were analyzed jointly to determine which were independent predictors of the fasting insulin level. Age, BMI, M, and MCRI were all highly significant ($P = 0.0016$, $P < 0.0001$, $P < 0.0001$, and $P = 0.0006$, respectively) predictors of fasting insulin in this joint analysis.

Fasting insulin and insulin resistance are known to be heritable traits (23). However, to our knowledge, the genetic contribution to MCRI has not been previously investigated. We used a variance component method to estimate the heritability of the clamp-derived indexes of insulin sensitivity and clearance (Table 4). In our population, the covariate-adjusted heritability of fasting insulin was 0.38 ($P = 0.0011$) and that of M was 0.44 ($P < 0.0001$). The heritability of S_i was 0.40. In comparison, the heritability of MCRI was substantially higher at 0.58 ($P < 0.0001$).

The frequencies of the nine polymorphic *AMPD1* SNPs are shown in Table 5. The genotype frequencies for all nine markers were in Hardy-Weinberg equilibrium. LD among the four markers (D′) ranged from 0.11 to 1.0 (average pairwise D′ of 0.86. Two haplotype blocks were identified, one major block spanning the 5′ end of the gene to intron 5 and a smaller haplotype block comprising the two SNPs in intron 6 (Fig. 2). The average pairwise LD within the 5′ haplotype block was 0.94.

The association of *AMPD1* SNPs with insulin-related traits was evaluated using QTDT. No SNP showed a significant association with fasting insulin or M. SNP3, SNP6, and SNP7 were associated with MCRI ($P = 0.037$, 0.041, and 0.0091, respectively). We also evaluated the association of haplotypes from the large haplotype block that extends for 14 kb from upstream of the *AMPD1* gene to intron 5. *AMPD1* haplotypes were not associated with fasting insulin or M. However, the most common haplotype, haplotype 1, and the second most common haplotype, haplotype 2, were both significantly associated with MCRI ($P = 0.017$ and 0.015, respectively). Of note, the minor alleles of the associated SNPs lie on these haplotypes, with those of SNP3 and SNP6 lying on haplotype 1 and that of SNP7 lying on haplotype 2 (Fig. 2).

Figure 3 shows the mean MCRI levels according to haplotype carrier status and haplogenotype among 320 individuals of the offspring generation who were haplotyped and phenotyped. Haplotype 1 was associated with increased MCRI and haplotype 2 was associated with decreased MCRI. A dosage-response relation was observed whereby the number of chromosomes bearing haplotype 1 corresponded with increasing MCRI, and the number of chromosomes bearing haplotype 2 corresponded with decreasing MCRI.

The heritability of MCRI was recalculated with the *AMPD1* haplogenotype as a covariate (Table 4) to assess the impact of the *AMPD1* genotype on the heritability of MCRI. In this model, the heritability of MCRI was 0.49 ($P < 0.0001$), indicating that the *AMPD1* genotype accounts for

TABLE 3
Correlations among variables of insulin metabolism and action

	Age	BMI	Fasting insulin	Glucose infusion rate	Metabolic clearance rate of insulin
Age	—	0.16*	−0.04	−0.13*	0.00023
BMI	0.017*	—	0.54*	−0.58*	−0.11*
Fasting insulin	0.68	<0.0001*	—	−0.55*	−0.33*
Glucose infusion rate	0.020*	<0.0001*	<0.0001*	—	0.085*
Metabolic clearance rate of insulin	0.75	0.016*	<0.0001*	0.032*	—

Data above the diagonal are *r* values; data below the diagonal are *P* values. *Significant value. *P* values were derived using generalized estimating equations to account for familial relationships.

TABLE 4
Heritability of indexes of insulin metabolism and action

	Without covariates		With covariates		
	$h^2 \pm$ SE	P	$h^2 \pm$ SE	P	Significant covariates
Fasting insulin	0.34 ± 0.13	0.0009	0.38 ± 0.14	0.0011	Age, BMI
Glucose infusion rate	0.41 ± 0.13	0.0002	0.44 ± 0.14	<0.0001	BMI
Metabolic clearance rate of insulin	0.57 ± 0.14	<0.0001	0.58 ± 0.14	<0.0001	BMI
Metabolic clearance rate of insulin	—	—	0.49 ± 0.14	<0.0001	BMI, *AMPD1* haplogenotype

The covariates taken into consideration were age, sex, and BMI. The *AMPD1* haplogenotype was included in the final model to assess the heritability of the metabolic clearance rate of insulin after adjusting for the *AMPD1* genotype.

~15% of the heritability of MCRI and that other as-yet unidentified genes must also contribute to the heritability of MCRI.

DISCUSSION

In this study, we examined the genetic nature of various insulin-related phenotypes. We found that MCRI is a highly heritable trait and that specific haplotypes in the *AMPD1* gene are closely linked to quantitative differences in the overall MCRI in our study population.

MCRI and M are independent predictors of fasting insulin concentration. Insulin-resistant nondiabetic subjects maintain normoglycemia by a compensatory increase in insulin secretion, which explains the negative correlation between insulin sensitivity and fasting insulin. In a similar vein, the negative correlation between MCRI and fasting insulin is consistent with the concept that once insulin clearance declines, insulin concentrations rise.

MCRI becomes of great interest because of the evidence presented herein that it is a highly heritable trait. In fact, it was more heritable in our study than M. To our knowledge, this is the first report assessing the heritability of MCRI. Insulin sensitivity/resistance is known to be heritable, as is evidenced by the observation of reduced insulin sensitivity in nondiabetic relatives of type 2 diabetic subjects (24,25). The heritability of insulin sensitivity is 0.28–0.44 when quantified by the frequently sampled intravenous glucose tolerance test (26,27) and is 0.37 when assessed by the euglycemic clamp (28). The heritability of M observed in our study is consistent with these reports.

As a major determinant of circulating insulin levels, MCRI is potentially of great importance in that insulin levels may play a role in modulating processes that influence the development of atherosclerosis. Proatherogenic effects of insulin include stimulation of proliferation of vascular smooth muscle cells, as well as production of plasminogen activator inhibitor 1 by these cells (29,30). In contrast, insulin may protect against atherosclerosis by antagonizing inflammatory transcription factors, inhibiting adhesion molecule expression, and promoting nitric oxide production in endothelial cells (31–33). The elucidation of genetic determinants of MCRI will provide insight into not only how insulin is cleared but also the mechanisms of insulin action.

TABLE 5
AMPD1 SNP frequencies

SNP	dbSNP designation	Genomic position	Nucleotide change (major allele → minor allele)	Minor allele frequency
1	rs926938	−1640	C→T	0.46
2	rs2010899	3167	T→G	0.22
3	rs2268701	5131	G→A	0.50
4	P48L	6923	C→T	0.060
5	rs2268698	10577	C→G	0.24
6	rs2268697	10684	C→T	0.45
7	rs3789627	13139	G→A	0.19
8	rs743041	15699	C→T	0.17
9	rs761755	15743	T→C	0.22

National Center for Biotechnology Information SNP database designation is not given for P48L (proline to leucine at amino acid 48). Genomic position is given with respect to the transcription start site.

The *AMPD1* gene (chromosome 1p13) codes for the muscle-specific form of the AMP deaminase enzyme (myoadenylate deaminase), which catalyzes the deamination of AMP to inosine monophosphate in skeletal muscle. Mutations in *AMPD1*, which are found in ~20% of the Caucasian population, are frequently found in patients with exercise-induced myopathy. Inherited defects in *AMPD1* that lead to decreased activity of this enzyme result in AMP accumulation in skeletal myocytes (7); the resultant alteration in adenylate energy charge has the potential to influence the activity of numerous enzymes. For example, a reduction in AMPD1 expression or function would lead to increased AMP levels, which activate AMPK.

AMPK serves as a cellular energy sensor, acting to maintain cellular ATP levels by phosphorylating metabolic enzymes and regulating gene expression (34). For example, AMPK phosphorylates and inactivates enzymes in the gluconeogenic pathway and inhibits gene expression of these enzymes (e.g., PEPCK, G6Pase) (35). AMPK stimulates muscle glucose uptake by increasing expression and translocation of GLUT4, stimulates fatty acid oxidation in muscle and liver, inhibits hepatic glucose production, and inhibits lipolysis and lipid synthesis (8,34,36). AMPK has emerged as a possible mediator of the effects of insulin-sensitizing medications (37,38). Of interest, biguanide and thiazolidinedione insulin sensitizers that activate AMPK also alter insulin clearance (39).

Alterations in AMPD1 activity, with its resultant effects on AMPK or other metabolic pathways, may not be uniformly manifest throughout the cell. AMPD1 binds reversibly to intracellular organelles, such as the myofibril (7); consequently, changes in the activity of this enzyme may alter metabolism differentially in localized regions of the myocyte. If the insulin receptor endocytic pathway, which is an energy-requiring, critical step in insulin clearance, was modified by local changes in the adenylate energy charge or if a protein component of this pathway were a substrate responsive to local changes in AMPK activity, then this might provide a mechanism for the

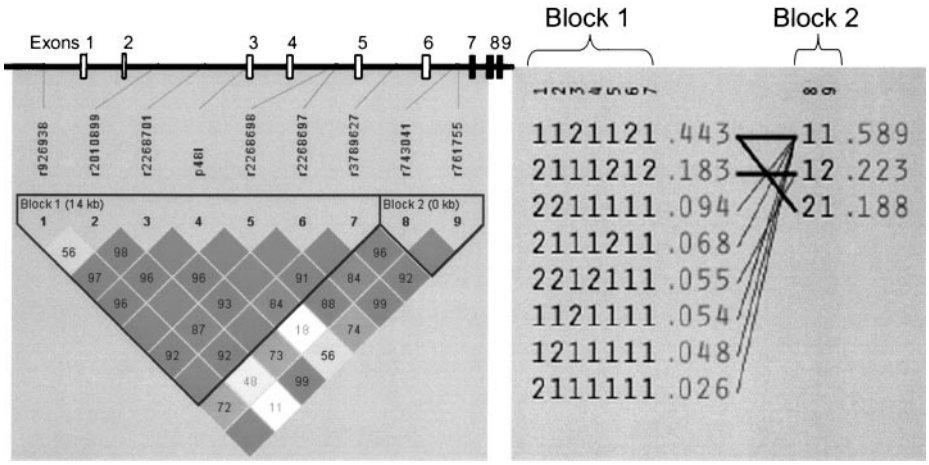

FIG. 2. LD plot showing haplotype blocks in the *AMPD1* gene. D′ values (percent) are indicated in the LD plot (*left panel*). The solid blocks indicate D′ = 1 (100%) for the corresponding pair of variants. *Right panel*: Haplotype listing (in rows) with corresponding population frequencies. Each row represents a haplotype, and each column represents one SNP (1 = major allele; 2 = minor allele). The program Haploview was used to generate this figure.

observed alterations of MCRI in patients with the various *AMPD1* genotypes.

AMPD1 is a tightly regulated, allosteric enzyme that contains unique regulatory domains in the nonconserved NH_2-terminal region that interact with the catalytic and nucleotide regulatory sites located in the conserved COOH-terminal region (7,40). Figures 1 and 2 indicate that the boundary between the two haplotype blocks observed maps closely to the boundary between the nonconserved, isoform-specific 5′ region of the *AMPD1* gene and the highly conserved 3′ region of this gene, which is shared with all members of this multigene family (7). Of note, the boundary between conserved and nonconserved region extends all the way to the yeast enzyme (41). The fact that we observed phenotype association of haplotypes only in the block that maps to the isoform-specific region of *AMPD1* further supports the conclusion that variation in this gene influences MCRI.

These results may find practical application in the pharmacokinetics of insulin treatment. When given as a drug, the clearance of administered insulin is an important determinant of the amount of insulin required to attain an appropriate plasma concentration. Thus, the *AMPD1* genotype may be one determinant of the insulin dosage required to achieve adequate glucose control in diabetic subjects.

It is important to note that, overall, in vivo measurements of insulin clearance primarily reflect the ability of the liver to extract and metabolize insulin. Renal excretion of insulin is also significant, and it has been estimated that together, hepatic and renal mechanisms account for up to 80% of total insulin clearance. Thus, skeletal muscle contributes a relatively small component of total insulin clearance, perhaps up to 20%, and changes in muscle insulin clearance will have only modest effects on total body insulin clearance. Because AMPD1 is a skeletal muscle−specific enzyme, any effect that a variation in AMPD1 expression or function has on insulin clearance must be exerted within skeletal muscle itself. With this line of reasoning, because the various measures of insulin clearance differ by 8–15% in patients with and without the *AMPD1* haplotype 1, it is possible to infer that this haplotype may lead to a 30–50% variation in skeletal muscle insulin clearance in affected individuals.

In summary, we have examined the genetic regulation of various insulin-related phenotypes. We found that the MCRI is a highly heritable trait and that specific haplotypes in the *AMPD1* gene are closely associated with quantitative differences observed in overall MCRI in our study group. AMPD1 is well located from a metabolic perspective to modulate other enzymes (e.g., AMPK) that are known to influence insulin action. The association we have described between *AMPD1* and insulin clearance provides insight into new biochemical pathways that can modulate insulin action and clearance in skeletal muscle, a critical target organ. Interventions that alter adenine nucleotide levels and adenylate energy charge may represent new therapeutic targets for modifying insulin action in syndromes of insulin resistance.

ACKNOWLEDGMENTS

The Mexican-American Coronary Artery Disease project is supported in part by National Institutes of Health Program

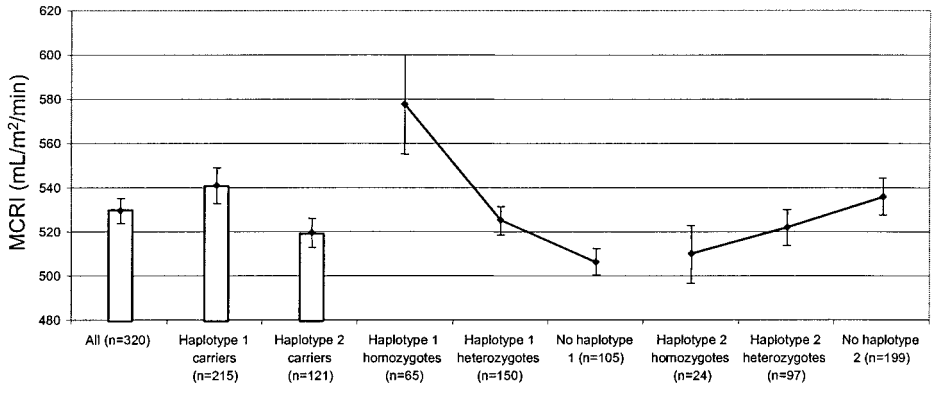

FIG. 3. Mean levels of insulin clearance by the *AMPD1* haplogenotype. The three bars represent the mean MCRI for all subjects, haplotype 1 carriers, and haplotype 2 carriers, respectively. The line plots represent the mean MCRI for the haplogenotypes indicated. Vertical lines represent the SE.

Project Grant HL-60030. Further support came from the Cedars-Sinai Board of Governors' Chair in Medical Genetics (to J.I.R.), the Cedars-Sinai General Clinical Research Center Grant RR000425, and the Diabetes Endocrinology Research Center Grant DK63491.

We thank all the study participants and referring physicians.

REFERENCES

1. Motulsky AG, Brunzell JD: Genetics of coronary atherosclerosis. In *The Genetic Basis of Common Diseases*, 2nd ed. King RA, Rotter JI, Motulsky AG, Eds. New York, Oxford University Press, 2002, p. 105–126
2. Park YW, Zhu S, Palaniappan L, Heshka S, Carnethon MR, Heymsfield SB: The metabolic syndrome: prevalence and associated risk factor findings in the US population from the Third National Health and Nutrition Examination Survey, 1988–1994. *Arch Intern Med* 163:427–436, 2003
3. Okosun IS, Liao Y, Rotimi CN, Prewitt TE, Cooper RS: Abdominal adiposity and clustering of multiple metabolic syndrome in white, black and Hispanic Americans. *Ann Epidemiol* 10:263–270, 2000
4. Haffner SM, Stern MP, Hazuda HP, Pugh J, Patterson JK, Malina R: Upper body and centralized adiposity in Mexican Americans and non-Hispanic whites: relationship to body mass index and other behavioral and demographic variables. *Int J Obes Relat Metab Disord* 10:493–502, 1986
5. DeFronzo RA, Tobin JD, Andres R: Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* 237:E214–E223, 1979
6. Wallace TM, Matthews DR: The assessment of insulin resistance in man. *Diabet Med* 19:527–534, 2002
7. Sabina RL, Holmes EW: Myoadenylate deaminase deficiency. In *The Metabolic and Molecular Bases of Inherited Disease*, 8th ed. Scriver CR, Beauder AL, Sly WS, Vall D, Eds. New York, McGraw-Hill, 2001, p. 2627–2638
8. Winder WW, Hardie DG: AMP-activated protein kinase, a metabolic master switch: possible roles in type 2 diabetes. *Am J Physiol* 277:E1−E10, 1999
9. Goodarzi MO, Guo X, Taylor KD, Quiñones MJ, Samayoa C, Yang H, Saad MF, Palotie A, Krauss RM, Hsueh WA, Rotter JI: Determination and use of haplotypes: ethnic comparison and association of the lipoprotein lipase gene and coronary artery disease in Mexican-Americans. *Genet Med* 5:322–327, 2003
10. Goodarzi MO, Guo X, Taylor KD, Quinones MJ, Saad MF, Yang H, Hsueh WA, Rotter JI: Lipoprotein lipase is a gene for insulin resistance in Mexican Americans. *Diabetes* 53:214–220, 2004
11. Toyama K, Morisaki H, Kitamura Y, Gross M, Tamura T, Nakahori Y, Vance JM, Speer M, Kamatani N, Morisaki T: Haplotype analysis of human AMPD1 gene: origin of common mutant allele. *J Med Genet* 41: e74, 2004
12. Gross M, Rotzer E, Kolle P, Mortier W, Reichmann H, Goebel HH, Lochmuller H, Pongratz D, Mahnke-Zizelman DK, Sabina RL: A G468-T AMPD1 mutant allele contributes to the high incidence of myoadenylate deaminase deficiency in the Caucasian population. *Neuromuscul Disord* 12:558–565, 2002
13. Morisaki T, Gross M, Morisaki H, Pongratz D, Zollner N, Holmes EW: Molecular basis of AMP deaminase deficiency in skeletal muscle. *Proc Natl Acad Sci U S A* 89:6457–6461, 1992
14. Livak KJ: Allelic discrimination using fluorogenic probes and the 5′ nuclease assay. *Genet Anal* 14:143–149, 1999
15. Zeger SL, Liang KY: Longitudinal data analysis for discrete and continuous outcomes. *Biometrics* 42:121–130, 1986
16. Almasy L, Blangero J: Multipoint quantitative-trait linkage analysis in general pedigrees. *Am J Hum Genet* 62:1198–1211, 1998
17. Barrett JC: *Haploview*: *Version 2.05 Edition*. Cambridge, MA, Whitehead Institute for Biomedical Research, 2004
18. Qin ZS, Niu T, Liu JS: Partition-ligation-expectation-maximization algorithm for haplotype inference with single-nucleotide polymorphisms. *Am J Hum Genet* 71:1242–1247, 2002
19. Abecasis GR, Cardon LR, Cookson WO: A general test of association for quantitative traits in nuclear families. *Am J Hum Genet* 66:279–292, 2000
20. Spielman RS, McGinnis RE, Ewens WJ: Transmission test for linkage disequilibrium: the insulin gene region and insulin-dependent diabetes mellitus (IDDM). *Am J Hum Genet* 52:506–516, 1993
21. Allison DB: Transmission-disequilibrium tests for quantitative traits. *Am J Hum Genet* 60:676–690, 1997
22. Abecasis GR, Cookson WO, Cardon LR: Pedigree tests of transmission disequilibrium. *Eur J Hum Genet* 8:545–551, 2000
23. Bergman RN, Zaccaro DJ, Watanabe RM, Haffner SM, Saad MF, Norris JM, Wagenknecht LE, Hokanson JE, Rotter JI, Rich SS: Minimal model-based insulin sensitivity has greater heritability and a different genetic basis than homeostasis model assessment or fasting insulin. *Diabetes* 52:2168–2174, 2003
24. Tripathy D, Lindholm E, Isomaa B, Saloranta C, Tuomi T, Groop L: Familiality of metabolic abnormalities is dependent on age at onset and phenotype of the type 2 diabetic proband. *Am J Physiol* 285:E1297−E1303, 2003
25. Volk A, Renn W, Overkamp D, Mehnert B, Maerker E, Jacob S, Balletshofer B, Haring HU, Rett K: Insulin action and secretion in healthy, glucose tolerant first degree relatives of patients with type 2 diabetes mellitus: influence of body weight. *Exp Clin Endocrinol Diabetes* 107:140–147, 1999
26. Hong Y, Weisnagel SJ, Rice T, Sun G, Mandel SA, Gu C, Rankinen T, Gagnon J, Leon AS, Skinner JS, Wilmore JH, Bergman RN, Bouchard C, Rao DC: Familial resemblance for glucose and insulin metabolism indices derived from an intravenous glucose tolerance test in blacks and whites of the HERITAGE Family Study. *Clin Genet* 60:22–30, 2001
27. Watanabe RM, Valle T, Hauser ER, Ghosh S, Eriksson J, Kohtamaki K, Ehnholm C, Tuomilehto J, Collins FS, Bergman RN, Boehnke M: Familiality of quantitative metabolic traits in Finnish families with non-insulin-dependent diabetes mellitus: Finland-United States Investigation of NIDDM Genetics (FUSION) Study investigators. *Hum Hered* 49:159–168, 1999
28. Lehtovirta M, Kaprio J, Forsblom C, Eriksson J, Tuomilehto J, Groop L: Insulin sensitivity and insulin secretion in monozygotic and dizygotic twins. *Diabetologia* 43:285–293, 2000
29. Avena R, Mitchell ME, Neville RF, Sidawy AN: The additive effects of glucose and insulin on the proliferation of infragenicular vascular smooth muscle cells. *J Vasc Surg* 28:1033–1038 (discussion 1038–1039), 1998
30. Pandolfi A, Iacoviello L, Capani F, Vitacolonna E, Donati MB, Consoli A: Glucose and insulin independently reduce the fibrinolytic potential of human vascular smooth muscle cells in culture. *Diabetologia* 39:1425–1431, 1996
31. Aljada A, Saadeh R, Assian E, Ghanim H, Dandona P: Insulin inhibits the expression of intercellular adhesion molecule-1 by human aortic endothelial cells through stimulation of nitric oxide. *J Clin Endocrinol Metab* 85:2572–2575, 2000
32. Aljada A, Ghanim H, Saadeh R, Dandona P: Insulin inhibits NFkappaB and MCP-1 expression in human aortic endothelial cells. *J Clin Endocrinol Metab* 86:450–453, 2001
33. Zeng G, Nystrom FH, Ravichandran LV, Cong LN, Kirby M, Mostowski H, Quon MJ: Roles for insulin receptor, PI3-kinase, and Akt in insulin-signaling pathways related to production of nitric oxide in human vascular endothelial cells. *Circulation* 101:1539–1545, 2000
34. Hardie DG, Hawley SA: AMP-activated protein kinase: the energy charge hypothesis revisited. *Bioessays* 23:1112–1119, 2001
35. Lochhead PA, Salt IP, Walker KS, Hardie DG, Sutherland C: 5-aminoimidazole-4-carboxamide riboside mimics the effects of insulin on the expression of the two key gluconeogenic genes PEPCK and glucose-6-phosphatase. *Diabetes* 49:896–903, 2000
36. Holmes BF, Kurth-Kraczek EJ, Winder WW: Chronic activation of 5′-AMP-activated protein kinase increases GLUT-4, hexokinase, and glycogen in muscle. *J Appl Physiol* 87:1990–1995, 1999
37. Fryer LG, Parbu-Patel A, Carling D: The anti-diabetic drugs rosiglitazone and metformin stimulate AMP-activated protein kinase through distinct signaling pathways. *J Biol Chem* 277:25226–25232, 2002
38. Zhou G, Myers R, Li Y, Chen Y, Shen X, Fenyk-Melody J, Wu M, Ventre J, Doebber T, Fujii N, Musi N, Hirshman MF, Goodyear LJ, Moller DE: Role of AMP-activated protein kinase in mechanism of metformin action. *J Clin Invest* 108:1167–1174, 2001
39. Iozzo P, Hallsten K, Oikonen V, Virtanen KA, Parkkola R, Kemppainen J, Solin O, Lonnqvist F, Ferrannini E, Knuuti J, Nuutila P: Effects of metformin and rosiglitazone monotherapy on insulin-mediated hepatic glucose uptake and their relation to visceral fat in type 2 diabetes. *Diabetes Care* 26:2069–2074, 2003
40. Gross M, Morisaki H, Morisaki T, Holmes EW: Identification of functional domains in AMPD1 by mutational analysis. *Biochem Biophys Res Commun* 205:1010–1017, 1994
41. Sabina R, Morisaki T, Clarke P, Eddy R, Shows T, Morton C, Holmes E: Characterization of the human and rat myoadenylate deaminase genes. *J Biol Chem* 265:9423–9433, 1990