Variants in *ACAD1O* are associated with type 2 diabetes, insulin resistance and lipid oxidation in Pima Indians

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Abstract

Aims/hypothesis—A prior genome-wide association study in Pima Indians identified a variant within the *ACAD10* gene that is associated with early-onset type 2 diabetes. Acyl-coenzyme A dehydrogenase 10 (ACAD10) catalyses mitochondrial fatty acid beta-oxidation, which plays a pivotal role in developing insulin resistance and type 2 diabetes. Therefore, *ACAD10* was analysed as a positional and biological candidate for type 2 diabetes.

Methods—Twenty-three SNPs were genotyped in 1,500 Pima Indians to determine the linkage disequilibrium pattern across *ACAD10*. Association with type 2 diabetes was determined by genotyping four tag single nucleotide polymorphisms (SNPs) in a population-based sample of 3,501 full-heritage Pima Indians; two associated SNPs were further genotyped in a second population-based sample of 3,723 American Indians. Associations with quantitative traits were assessed in 415 non-diabetic full heritage Pima individuals who had been metabolically phenotyped.

Results—SNPs rs601663 and rs659964 were associated with type 2 diabetes in the full-heritage Pima Indian sample (*p*=0.04 and 0.0006, respectively), and rs659964 was further associated with type 2 diabetes in the second American Indian sample (*p*=0.04). Combination of these two samples provided the strongest evidence for association (*p*=0.009 and 0.00007, for rs601663 and rs659964, respectively). Quantitative trait analyses identified nominal associations with both lower lipid oxidation rate and larger subcutaneous abdominal adipocyte size, which is consistent with the known physiology of *ACAD10*, and also identified associations with increased insulin resistance.

Conclusions/interpretation—We propose that *ACAD10* variation may increase type 2 diabetes susceptibility by impairing insulin sensitivity via abnormal lipid oxidation.

Keywords

Acyl-coenzyme A dehydrogenase 10; Association; Insulin resistance; Linkage disequilibrium; Lipid oxidation; Pima Indians; Type 2 diabetes

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Introduction

To identify genes that contribute to type 2 diabetes among Pima Indians, a population with an extremely high prevalence of this disease [1], we previously completed a genome-wide association study (GWAS) using the Affymetrix 100 K array [2]. Approximately 30 genes that mapped near the strongest GWAS signals for diabetes and had a plausible physiological role in a diabetes-related process were prioritised for detailed follow-up analyses. One of these genes was the *ACAD10* gene. A single nucleotide polymorphism (SNP), rs632650, that maps within intron 2 of *ACAD10* ranked among the top 1% for association with early-onset type 2 diabetes (defined as diabetes onset at age <25 years) in the GWAS. The acyl-coenzyme A dehydrogenase 10 (ACAD10) protein product catalyses the mitochondrial beta-oxidation of fatty acyl-CoA derivatives, and it has been suggested that disordered mitochondrial fatty acid oxidation induces insulin resistance in liver and skeletal muscle by promoting increased intracellular lipid metabolites [3]. Therefore, *ACAD10* was analysed as a positional and biological candidate gene for type 2 diabetes in Pima Indians.

Methods

Study participants

All participants were part of our longitudinal study of the aetiology of type 2 diabetes among the Gila River Indian Community in Arizona [1]. Informed consent was obtained from all participants and studies were approved by the tribal council of the Gila River Indian Community and the institutional review board of the National Institute of Diabetes and Digestive and Kidney Diseases. Association with type 2 diabetes was initially assessed in a population-based sample of 3,501 full-heritage Pima Indians (median age at last examination 38 years), of whom 1,561 had been diagnosed with type 2 diabetes (mean age 48.5±14.1 years; 37% male) and 1,940 were non-diabetic at the time of their last exam (mean age 31.1±14.5 years; 46% male). A subsequent replication study was performed in a second population-based sample of 3,723 American Indians (median age at last examination 23 years), most of whom were of mixed heritage (on average their reported heritage was one-half Pima Indian and three-quarters American Indian). This American Indian sample consisted of 750 individuals who had been diagnosed with type 2 diabetes (mean age 42.5±14.2 years; 41% male) and 2,973 individuals who were non-diabetic (mean age 23.6±10.9 years; 47% male) at the time of their last exam. Associations with diabetes-related quantitative traits were examined in a subgroup of 415 non-diabetic full-heritage Pima Indians (mean age 26.7±6.2 years; 58% male) who were determined to be healthy and then underwent detailed metabolic testing.

Metabolic phenotyping

A 75 g OGTT was used to determine diabetes status, according to the criteria of the World Health Organization. Body composition was estimated by underwater weighing or by total body dual energy x-ray absorptiometry (DPX-1; Lunar Radiation, Madison, WI, USA). The two-step hyperinsulinaemic-euglycaemic clamp technique was used to determine insulin-stimulated glucose disposal rates as previously described [4]. Ventilated-hood indirect calorimetry was used to estimate rates of lipid and glucose oxidation. The subcutaneous abdominal fat biopsies and the assessment of adipocyte size have been described elsewhere [5]. Basal lipolysis was determined by measuring glycerol release from the isolated adipocytes as previously described [6].

Sequencing and genotyping

Exons, exon–intron boundaries and 2 kb of the putative promoter region were sequenced in 24 Pima Indians. In addition to the five variants identified by sequencing and the lead SNP from the previous GWAS, 17 additional database SNPs were selected from National Center for

Biotechnology Information (NCBI) dbSNP to provide coverage across a 65 kb region that spanned the entire *ACAD10* locus and the 5′ region of the *BRAP* gene. These 23 SNPs were genotyped in 1,500 full-heritage Pima Indians to determine the linkage disequilibrium (LD) structure of *ACAD10*. Four tag SNPs were subsequently genotyped in the full-heritage Pima Indian population and two tag SNPs were further genotyped in the second American Indian sample. Genotyping was performed by SNPlex (Applied Biosystems, Foster City, CA, USA), following the manufacturer's protocol.

Statistical analysis

Analyses were performed using SAS software version 9.1 (SAS Institute, Cary, NC, USA). Logistic regression was used to assess the association between genotypes and diabetic status, whereas linear regression was used for the association analysis between quantitative traits and genotypes. Both logistic and linear models were fit with the generalised estimating equation procedure to account for the family membership and adjust for other potential confounding variables. In the second population sample of mixed-heritage individuals, the individual estimate of European admixture was also used as a covariate based on 39 markers with large difference in allele frequency between populations [7]. LD patterns were determined by Haploview, version 3.32 (Broad Institute, Cambridge, MA, USA) and tag SNPs were selected with a pairwise $r^2 \geq 0.8$ taken as indicative of redundancy; the method of Gabriel et al. [8] was used to define haplotype blocks. All 23 SNPs were contained in a single haplotype block, and all common haplotypes (frequency >0.01) could be defined by four tag SNPs. Analyses of the associations of these haplotypes with the traits of interest were performed with a modification of the zero-recombinant haplotyping procedure, as previously described [9].

Results and discussion

HapMap data from the Chinese (Han Chinese in Beijing; CHB), which is generally the most similar to Pima Indians, was initially used to estimate the LD block structure around the GWAS lead SNP rs632650. The block containing this diabetes-associated SNP extended 65 kb and encompassed the entire *ACAD10* locus and the 5′ region of the adjacent *BRAP* gene. To verify that this region is also in high LD in Pima Indians, 23 SNPs that included five variants (none novel) detected by sequencing *ACAD10* in Pima Indians as well as additional database SNPs that covered non-sequenced genomic regions spanning *ACAD10* and the 5′ region of *BRAP* were genotyped in 1,500 full-heritage Pima Indians (SNPs detailed in Electronic supplementary material [ESM] Table 1). Similar to the Chinese data, all 23 SNPs fell into one haplotype block in Pima Indians and four genotypic groups could be established by grouping SNPs with an $r^2 \geq 0.98$ (ESM Fig. 1). Four tag SNPs (rs7136874, rs601663, rs659964 and rs7132509) representing each genotypic group were genotyped in 3,501 full-heritage Pima Indians for type 2 diabetes association analysis (Table 1). Rs659964 (group 3) had the strongest association with type 2 diabetes ($p=0.0006$, OR 1.28 per copy of the G allele); this SNP was highly concordant with the GWAS lead SNP rs632650. In addition, rs601663 (group 2) also showed modest association with type 2 diabetes in the full-heritage Pima Indians ($p=0.04$, OR 1.14 per copy of the C allele). To assess whether the association with type 2 diabetes could be replicated in a separate group of individuals, both rs659964 and rs601663 were further genotyped in a second population-based sample of 3,723 American Indians, most of whom were of mixed heritage (Table 1). The association of rs659964 with type 2 diabetes nominally replicated in the second sample ($p=0.04$, OR 1.20), whereas rs601663 showed only a non-significant trend ($p=0.11$, OR 1.13). To increase statistical power, we further analysed the combined full-heritage and American Indian samples that provided the strongest evidence for association with type 2 diabetes ($p=0.00007$, OR 1.25 and 0.009, OR 1.14 for rs659964 and rs601663, respectively; Table 1).

These tag SNPs were also analysed for association with rype-2-diabetes-related traits among 415 non-diabetic full-heritage Pima Indians (Table 2). Rs659964 and rs7136874 were associated with reduced post-absorptive lipid oxidation rates (p=0.02), as assessed using ventilated-hood indirect calorimetry. The alleles associated with lower post-absorptive lipid oxidation rates were further associated with larger isolated adipocytes, even after adjusting for percentage of body fat of the donor. Significantly reduced basal lipolysis rates within isolated adipocytes were further observed for rs7136874 (p=0.01). In addition, the diabetic risk alleles for rs7136874 and rs601663 were also associated with lower in vivo insulin-stimulated glucose disposal rates (insulin resistance, p=0.05 and 0.01, respectively) during a clamp, as well as elevated 2 h plasma insulin levels in response to an OGTT (p = 0.005 and 0.003, respectively). Our results of the quantitative trait analyses suggest that variation in *ACAD10* might induce insulin resistance via impaired lipid oxidation and/or enlarged adipocytes. Both decreased post-absorptive lipid oxidation and the reduced lipolysis within the adipocytes may accelerate triacylglycerol accumulation, therefore providing a plausible mechanism for the enlarged adipocyte size, which has previously been correlated with insulin resistance and risk for developing type 2 diabetes [5].

To determine whether a specific haplotype across *ACAD10* provided stronger evidence for association than a single SNP alone, an exhaustive haplotype analysis of all possible combinations of tag SNPs (ESM Table 2) was performed. No haplotype provided stronger evidence for association with diabetes than rs659964 alone, and the significant haplotypes were highly concordant with rs659964, suggesting that the associations largely reflected the single-marker association of rs659964. Similarly, exhaustive haplotype analysis for each of the quantitative traits suggested that the single SNP analyses presented in Table 2 reflected the strongest association for each trait.

Although the association of *ACAD10* with type 2 diabetes and lipid oxidation is supported by the known biological function of this gene, it should be cautioned that multiple comparisons can lead to false-positive associations and that these data were not corrected for multiple testing. A Bonferroni correction for four tag SNPs and ten traits would render all of the associations with quantitative traits non-significant However, replication of associations in other populations can also provide evidence that associations are not spurious. Among the 23 SNPs initially genotyped in Pima Indians (ESM Table 1), 14 were also analysed for association with type 2 diabetes in the DIAGRAM [10] meta-analysis of Europeans (ESM Table 3). Five SNPs showed nominal associations (p<0.05) with type 2 diabetes in the DIAGRAM study, and these SNPs fell into Pima groups 2 and 3 (represented by rs601663 and rs659964, respectively). However, among the associated SNPs, the European risk alleles were consistently the Pima non-risk alleles. Four of the five SNPs associated with type 2 diabetes in the Europeans have also been analysed for association with glycaemic traits in Europeans as part of the MAGIC consortium. Two of these SNPs (rs632650 and rs659964) were associated with 2 h plasma glucose levels, adjusted for BMI, among 15,234 individuals (p=0.03 and 0.04, respectively), in which the glucose-increasing alleles were consistent with the diabetes risk alleles reported in DIAGRAM. As the haplotype structure and SNP frequencies differ in Pima Indians vs Europeans (the major/minor alleles for SNPs in group 2 are flipped in Pima vs Europeans, but are consistent in group 3, whereas many of the group 3 SNPs in Pima Indians are tagged by group 1 SNPs in Europeans), it remains unclear whether finding a consistent association of the same SNP with both diabetes and a glycaemic trait in each population separately, where the risk alleles are flipped between ethnic groups, simply occurred by chance or whether an unidentified causative variant underlies these associations in both ethnic groups.

At present, it appears unlikely that any of the identified variants are causative, because none are in a conserved region/motif and, owing to the high degree of LD, there are presumably dozens of untyped variants that will have similar associations. In addition, although the known

function of *ACAD10* is consistent with the observed lipid oxidation associations, the high LD across this region, which also contains the 5′ region of the *BRAP* gene, does not allow us to conclude unambiguously that *ACAD10* is the true causative gene.

In conclusion, we suggest that *ACAD10* variation may increase susceptibility to type 2 diabetes and that the effect may be mediated by impairing insulin sensitivity via abnormal lipid oxidation, but deep re-sequencing and functional studies are required to confirm this hypothesis.

Abbreviations

ACAD10	Acyl-coenzyme A dehydrogenase 10
GWAS	Genome-wide association study
LD	Linkage disequilibrium
SNP	Single nucleotide polymorphism

Acknowledgments

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Table 1

Association of *ACAD10* tag SNPs with type 2 diabetes in the full-heritage Pima Indian, second American Indian and combined population sets

Genotypic group	SNP	Allele (1/2)	Full-heritage Pima Indian population-based sample (1,561 diabetic + 1,940 non-diabetic)			American Indian population-based sample (750 diabetic + 2,973 non-diabetic)			Combined samples	
			Allele 2 (%) (diabetic/non-diabetic)	p value	OR (95% CI)	Allele 2 (%) (diabetic/non-diabetic)	p value	OR (95% CI)	p value	OR (95% CI)
1	rs7136874	C/T	0.47/0.45	0.14	1.09 (0.97–1.22)	–	–	–	–	–
2	rs601663	T/C	0.69/0.65	0.04	1.14 (1.01–1.29)	0.62/0.60	0.11	1.13 (0.97–1.31)	0.009	1.14 (1.03–1.25)
3	rs659964	C/G	0.23/0.20	0.0006	1.28(1.11–1.49)	0.21/0.18	0.04	1.20(1.01–1.43)	0.00007	1.25(1.12–1.41)
4	rs7132509	G/A	0.76/0.75	0.16	1.11 (0.96–1.27)	–	–	–	–	–

For each SNP, the frequency of allele 2 was given in the form of 'diabetic/non-diabetic', and ORs were expressed as per copy of allele 2 *p* values were given for the additive model and were adjusted for age, sex, birth year and Pima heritage

Table 2

Association of *ACAD10* tag SNPs with diabetes-related traits among non-diabetic full-heritage Pima Indians

Variable	rs7136874 (1=C, 2=T)				rs601663 (1=T, 2=C)				rs659964 (1=C, 2=G)				rs7132509 (1=G, 2=A)			
	11	12	22	p value	11	12	22	p value	11	12	22	p value	11	12	22	p value
Whole body measures																
Male/female (n)	70/43	102/76	52/48		26/16	109/64	88/83		142/90	62/59	9/12		15/13	91/59	117/93	
Age (years)	28 (7)	26 (5)	27 (6)		29 (6)	26 (6)	27 (6)		27 (6)	26 (6)	26 (5)		27 (6)	26 (6)	27 (6)	
Percentage of body fat[a,b,c]	33 (9)	33 (8)	33 (8)	0.51	32 (9)	33 (8)	33 (8)	0.77	33 (9)	33 (8)	34 (8)	0.33	34 (9)	33 (8)	33 (9)	0.79
Basal lipid oxidation (mg [kg EMBS]$^{-1}$ min^{-1})[a,b,c,d]	0.71 (0.23)	0.75 (0.24)	0.64 (0.24)	0.02	0.65 (0.23)	0.76 (0.24)	0.69 (0.24)	0.33	0.73 (0.22)	0.70 (0.26)	0.59 (0.18)	0.02	0.72 (0.21)	0.71 (0.25)	0.72 (0.24)	0.81
Basal carbohydrate oxidation (mg [kg EMBS]$^{-1}$ min^{-1})[a,b,c,d]	1.39 (0.44)	1.39 (0.45)	1.48 (0.46)	0.16	1.48 (0.52)	1.37 (0.44)	1.44 (0.44)	0.76	1.37 (0.43)	1.48 (0.47)	1.53 (0.40)	0.01	0.31 (0.38)	1.42 (0.47)	1.42 (0.44)	0.40
Fasting plasma glucose (mmol/l)[a,b,c,d]	5.01 (0.49)	4.97 (0.54)	5.00 (0.57)	0.89	5.08 (0.59)	4.96 (0.49)	5.00 (0.59)	0.67	5.00 (0.54)	4.99 (0.53)	5.00 (0.55)	0.90	5.05 (0.59)	4.97 (0.56)	5.00 (0.52)	0.87
2 h plasma glucose (mmol/l)[a,b,c,d]	6.73 (1.61)	6.79 (1.68)	7.05 (1.69)	0.15	7.00 (1.75)	6.60 (1.64)	7.00 (1.67)	0.38	6.71 (1.66)	7.03 (1.68)	7.25 (1.70)	0.03	6.97 (1.97)	6.72 (1.56)	6.87 (1.69)	0.83
Log (fasting plasma insulin [pmol/l])[a,b,c,d]	2.38 (1.06)	2.41 (1.05)	2.41 (1.05)	0.05	2.40 (1.08)	2.39 (1.05)	2.42 (1.04)	0.18	2.41 (1.06)	2.40 (1.03)	2.37 (1.04)	0.97	2.45 (1.09)	2.41 (1.04)	2.39 (1.05)	0.09
Log (2 h plasma insulin [pmol/l])[a,b,c,d]	3.00 (1.16)	3.03 (1.19)	3.09 (1.18)	0.005	3.00 (1.20)	2.99 (1.17)	3.10 (1.17)	0.003	3.02 (1.18)	3.08 (1.18)	3.00 (1.19)	0.25	3.14 (1.15)	3.04 (1.20)	3.02 (1.16)	0.05
Log (insulin stimulated glucose disposal [mg (kg EMBS)$^{-1}$ min^{-1}])[a,b,c,d]	0.55 (0.12)	0.55 (0.12)	0.53 (0.12)	0.05	0.56 (0.14)	0.55 (0.12)	0.53 (0.11)	0.01	0.55 (0.12)	0.54 (0.10)	0.55 (0.10)	0.26	0.51 (0.15)	0.55 (0.12)	0.55 (0.11)	0.43
Isolated adipocytes																
Male/female (n)	26/20	34/46	21/23		7/6	40/36	35/45		48/43	26/35	3/7		5/6	34/29	42/52	
Age (years)	27 (6)	26 (5)	26 (6)		28 (6)	26 (6)	26 (5)		27 (6)	25 (5)	26 (6)		25 (4)	26 (6)	26 (6)	
Average cell size (μg lipid/cell)[a,b,c,d]	0.73 (0.20)	0.77 (0.22)	0.83 (0.20)	0.02	0.78 (0.25)	0.72 (0.20)	0.83 (0.21)	0.03	0.76 (0.20)	0.81 (0.22)	0.83 (0.23)	0.06	0.79 (0.19)	0.80 (0.21)	0.76 (0.21)	0.66

Variable	rs7136874 (1=C, 2=T)			rs601663 (1=T, 2=C)			rs659964 (1=C, 2=G)			rs7132509 (1=G, 2=A)		
	11	12	22	p value	11	12	22	p value	11	12	22	p value	11	12	22	p value
Basal lipolysis rate (fmol cell^{-1} s^{-1}) [a,b,c,d]	0.14 (0.08)	0.13 (0.07)	0.11 (0.07)	0.01	0.15 (0.09)	0.12 (0.07)	0.13 (0.07)	0.30	0.13 (0.08)	0.12 (0.07)	0.12 (0.07)	0.24	0.12 (0.09)	0.11 (0.07)	0.13 (0.07)	0.10

For each trait, the raw mean is shown with the corresponding SD in parentheses

Plasma insulin concentrations (at fasting and 2 h) and insulin-stimulated glucose disposal rates during the clamp were log10-transformed before analyses to approximate a normal distribution Alleles 1 and 2 are as shown in Table 1

p values were adjusted for the covariates as indicated: [a]age; [b]sex; [c]family membership; [d]percentage of body fat

EMBS, estimated metabolic body size