Research article

The A54T polymorphism at the intestinal fatty acid binding protein 2 is associated with insulin resistance in glucose tolerant Caucasians

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Abstract

Background: An A54T polymorphism at the fatty acid binding protein 2 (FABP2) locus was found to be associated with insulin resistance in non-diabetic Pima Indians. To see whether this association is present in other populations, we performed a cross sectional study to examine the role of this polymorphism on insulin resistance in 55 healthy and normotensive Caucasian subjects with normal glucose tolerance. Insulin sensitivity (%S) and beta cell function (%B) were assessed using the Homeostasis Model Assessment (HOMA). Their genotypes were determined using a polymerase chain reaction-restriction fragment length polymorphism assay. The relationship between the genotypes and the phenotypes was examined.

Results: After genotyping, we identified 24 AA, 27 AT and 4 TT subjects. The TT subjects were combined with the AT subjects during the analysis due to its small sample size. No differences were noted in gender distribution, clinical features, and fasting lipid profile between the two genotypic groups (AA vs. AT/TT). The AT/TT group had a higher fasting plasma insulin concentration and a lower %S than the AA group ($p = 0.0444$ and $p = 0.0461$, respectively). However, no differences were noted in plasma glucose concentrations and %B. Univariate analysis revealed that this polymorphism explained 7.3% of the variation in %S. Multivariate analysis revealed that the polymorphism was an independent determinant for %S ($p = 0.0434$) and with body mass index accounted for 28.7% of the variation in %S. In contrast, this polymorphism had no impact on %B.

Conclusions: The A54T polymorphism at the FABP2 locus is a risk factor for insulin resistance in a Caucasian population.

Introduction

The Pima Indians have a very high prevalence for type 2 diabetes mellitus (or non-insulin-dependent diabetes mellitus, NIDDM) with evidence of strong familial aggregation [1]. In this population, insulin resistance is a major risk factor for the development of the disease [2], and maximal insulin action (i.e. glucose disposal rate at pharmacological insulin levels) was found to be determined by a co-dominantly inherited autosomal gene [3]. Initially, Bogardus and colleagues observed an association and linkage between insulin resistance and red cell antigens on chromosome 4q [4]. After the analysis of 128 sib-pairs using quantitative trait sib-pair analysis, they observed a significant linkage between maximal insulin action and the intestinal fatty acid-binding protein 2 (FABP2) gene and the annexin V (ANX5) gene on chromosome 4q [5].

It is well recognized that fatty acid metabolism is linked to insulin resistance [6,7]. Intestinal FABP2 contains a single ligand binding site that displays a high affinity for fatty acid [8]. Because it is a candidate gene at this locus, a search for a mutation was initiated and an Alanine (GCT) to Threonine (ACT) polymorphism at codon 54 was identified in Pima Indians [9]. The associations between this polymorphism and fasting insulin concentration, fasting fat oxidation, and glucose uptake during a hyperinsulinemic euglycemic clamp were identified in 137 non-diabetic Pima Indians [9].

Because NIDDM is a genetic disorder [10] and results from an imbalance between insulin sensitivity and beta cell function, we hypothesized that the A54T polymorphism of the FABP2 gene plays a role in the pathogenesis of insulin resistance, which is one of the key determinants for the development of NIDDM [2]. Since insulin sensitivity is affected by hypertension [11,12] and abnormal glucose tolerance [2], we examined the relationship of this polymorphism with insulin sensitivity in 55 healthy and normotensive Caucasians with normal glucose tolerance.

Results

The clinical features of the studied subjects were shown in Table 1. Using the PCR-RFLP assay, we identified 24 AA, 27 AT, and 4 TT subjects. In this Caucasian population, the allele frequency was 68% for the A allele and 32% for the T allele. The distribution of genotypes was in compliance with the Hardy-Weinberg equilibrium (p = 0.8321).

Since there were only 4 TT subjects, they were pooled with the AT subjects during the analysis. There were no differences in clinical features between the two genotypic groups (AA vs. AT/TT) as shown in Table 2. Although the AT/TT subjects had a higher fasting plasma insulin concentration than the AA subjects (p = 0.0444), no differences were noted in fasting plasma glucose concentrations and postchallenged plasma glucose and insulin concentrations. We estimated insulin sensitivity (%S) and beta cell function (%B) using the average of the three fasting plasma glucose and insulin concentrations. While no difference was noted in %B, the AT/TT subjects were more insulin resistant (a lower %S) than the AA subjects (p = 0.0461).

Since a higher body mass index had been reported to be associated with this polymorphism [13], we were concerned that the observed differences could be the result of other confounding covariates. A multivariate analysis was performed to examine the influence of this polymorphism and other covariates (Table 3). This polymorphism and body mass index explained 28.7% of the variation in %S and this polymorphism was an independent determinant of %S (p = 0.0434). However, this polymorphism had no impact on %B. Age, gender and waist hip ratio accounted for 19.5% of the variation in %B.

Table 1: Clinical features of the studied subjects

		Mean* (n)	Std. Dev.	Minimum	Maximum
N		55			
Gender	F/M	29/26			
Age	year	28	6	20	39
Body mass index	kg/m^2	24.52	3.87	17.58	34.26
Waist-hip ratio	cm/cm	0.81	0.09	0.65	1.03
Systolic blood pressure	mmHg	114	10	94	137
Diastolic blood pressure	mmHg	68	7	55	83
Oral glucose tolerance test					
Fasting plasma glucose	mM	4.72	0.35	3.88	5.55
Plasma glucose at 30 minutes	mM	7.44	1.27	5.49	9.66
Plasma glucose at 60 minutes	mM	7.14	1.44	4.44	10.20
Plasma glucose at 90 minutes	mM	6.30	1.29	3.62	9.02
Plasma glucose at 120 minutes	mM	5.98	1.06	2.94	7.60

* arithmetic means

Table 2: Clinical features and glycemic parameters by the FABP2 genotypes

		AA		AT/TT	
		Mean (n)	(95% CI)	Mean (n)	(95% CI)
N		24		31	
Gender	F/M	15/9		14/17	
Age	year	28	(26,31)	27	(25,29)
Body mass index[1]	kg/m^2	23.99	(22.52,25.56)	24.42	(23.03,25.90)
Waist-hip ratio[1]	cm/cm	0.80	(0.76, 0.84)	0.80	(0.77, 0.83)
Systolic blood pressure	mmHg	112	(109,116)	116	(112,120)
Diastolic blood pressure	mmHg	68	(65,71)	68	(65,70)
Triglycerides	mg/dL	89	(66,110)	73	(57,89)
Total cholesterol	mg/dL	166	(152,180)	152	(141,163)
HDL cholesterol	mg/dL	50	(45,54)	48	(43,54)
LDL cholesterol	mg/dL	98	(85,111)	89	(79,99)
Oral glucose tolerance test					
Fasting plasma glucose	mM	4.68	(4.53, 4.83)	4.75	(4.62,4.89)
Fasting plasma insulin[1,2]	pM	55	(48,63)	65	(59,72)
%B[1]		139	(118,163)	150	(132,171)
%S[1,3]		0.63	(0.54, 0.73)	0.52	(0.47, 0.58)

[1] geometric means; [2] $p = 0.0444$; [3] $p = 0.0461$.

Discussions

In this study, we found that the A54T polymorphism of the FABP2 was associated with insulin resistance and accounted for 7.3% of the variation in %S. Multivariate analysis confirmed that this polymorphism was an independent risk factor for insulin resistance. In contrast, this polymorphism had no impact on %B. Our observations confirm that the A54T polymorphism of the FABP2 affects insulin sensitivity, which was previously reported in Pima Indian and Japanese populations [9,14].

The FABP2 locus has been studied extensively. MNS red cell antigens, one of the few genetic markers available at that time, were initially found to be linked to insulin resistance in 86 non-diabetic Pima Indians from 31 nuclear families using a sib-pair analysis and they were also associated with insulin resistance in 132 non-diabetic Pima Indians [4]. With additional families and additional genetic markers, a significant linkage was identified between the FABP2 and ANX5 loci on chromosome 4q with insulin resistance in 123 non-diabetic subjects from 46 nuclear families [5]. Furthermore, the linkage of the FABP2 locus with insulin resistance (2-hour postchallenged insulin concentration) was also found in a Mexican-American population, which is genetically related to the Pima Indians [15]. However, sib-pair analysis failed to detect any linkage of the FABP2 locus or the A54T polymorphism with diabetes related phenotypes in other ethnic groups [16,17] as shown in Table 4A. Furthermore, no linkage was found between the FABP2 locus (Table 4B) and diabetes [18,19], lipodystrophic diabetes [20], or obesity [17]. As shown in Table 4C, the population association studies of the FABP2 locus with diabetes/impaired glucose tolerance were negative in UK, Finnish, and Welsh [21] and Japanese populations [22]. Subsequently, the A54T polymorphism was identified and was found to be associated with increased fatty acid binding protein, increased fat oxidation and increased insulin resistance in the Pima Indians [9]. The population association studies of the A54T polymorphism with NIDDM [9,23–25], coronary artery disease [26,27], obesity [24], and hypertension [24] were also essentially negative. Positive associations were mostly observed in quantitative association studies as shown in Table 4D. In addition to the original positive association between the A54T polymorphism and fasting plasma insulin concentration, fasting fat oxidation and glucose uptake in non-diabetic Pima Indians [9], positive association was also found with obesity [13,14], 2-hour post-challenged insulin concentration [14], and various lipid metabolism [13,28–30]. In the present study, we also demonstrated a positive quantitative association between this polymorphism and %S. However, numerous negative studies were also noted [23,24,29,31–33].

Table 3: Multivariate analysis

Dependent Variable	Covariate Entered	Covariate Removed	r^2	P
%S			0.287	
	Body mass index			0.0002
	FABP2 polymorphism			0.0434
		Waist-hip ratio		0.1045
		Gender		0.5313
		Systolic blood pressure		0.8157
		Age		0.8560
		Diastolic blood pressure		0.8909
%B			0.195	
	Age			0.0109
	Gender			0.0169
	Waist-hip ratio			0.0671
		Systolic blood pressure		0.2636
		Body mass index		0.2684
		FABP2 polymorphism		0.3215
		Diastolic blood pressure		0.3371

Clearly there is substantial controversy surrounding this locus and the A54T polymorphism in the pathogenesis of insulin resistance and T2DM as described above. Disagreement also occurred in the original Pima Indian study. Although significant linkages were identified at the FABP2 locus with fasting insulin concentration ($p = 0.0004$) and with glucose uptake ($p = 0.0008$) in the Pima Indians [5], the differences regarding the A54T polymorphism with fasting insulin concentrations ($p < 0.04$) and glucose uptake ($p < 0.04$) were only marginally significant in the same population [9]. The significant linkage in the original sib-pair study [5] could be the combined results of a highly polymorphic marker of the FABP2 locus (5 alleles vs. 2 alleles for the A54T polymorphism) and less intrafamilial difference in insulin sensitivity as the result of familial clustering of insulin sensitivity. In contrast, the marginal difference reflected a very modest influence on insulin sensitivity of the A54T polymorphism in the Pima Indian population, which is consistent with our observation that this polymorphism only accounted for 7.3% of the variation in %S in this Caucasian population. Furthermore, since 1) insulin resistance is neither necessary nor sufficient for the development of T2DM, 2) this polymorphism has only a very modest influence on insulin sensitivity, and 3) beta cell dysfunction, on which this polymorphism has no influence in the present study, plays a key role in the development of overt diabetes [2], the population association studies and linkage studies are not able to detect the interaction between this polymorphism and the diabetes phenotype. In contrast, quantitative studies of diabetes-related phenotypes become more rewarding in detecting a polymorphism of a very modest effect as shown in Table 4D. The negative quantitative studies could be the result of other confounding factors, such as the inclusion of diabetic, impaired glucose tolerant or hypertensive subjects in the study. Therefore, we only enrolled glucose tolerant and normotensive subjects in the present study for the reasons described previously.

The most convincing evidence that supports the A54T polymorphism as a causal mutation is from a functional study of mutated FABP2 [9]. FABP2 plays a role in the absorption and intracellular transportation of dietary long-chain fatty acids [8]. Thr54-containing FABP2 has a twofold greater affinity for long-chain fatty acids than the Ala54-containing FABP2 [9]. As predicted by the proposed [6,7] and subsequently proven [34] "Randle's cycle", an increased concentration of fatty acid inhibits glucose uptake in muscle and results in insulin resistance. Furthermore, two interventional studies showed that this A54T polymorphism affected lipid metabolism during interventions [35,36], as shown in Table 4E.

In summary, we examined the role of the A54T polymorphism in the pathogenesis of insulin resistance in 55 glucose tolerant and normotensive healthy Caucasians. We found that this polymorphism had an independent, but very modest influence (7.3%) on insulin sensitivity (%S), which was assessed by the HOMA. However, it had no impact on beta cell function (%B). To our knowledge, this is the very first report of a positive association between this polymorphism and insulin sensitivity in a Caucasian population.

Table 4: Published studies of FABP2 and A54T

Subjects/description	Genetic marker	Result*	Phenotype	Ref
A. Sib-pair study				
Non-diabetic Pima Indians				[4]
	MNS red cell antigen	+	maximal insulin-stimulated glucose uptake	
		+	insulin action index	
		-	fasting insulin	
Non-diabetic Pima Indians				[5]
	ANX5	+	maximal insulin-stimulated glucose uptake	
	FABP2	+	maximal insulin-stimulated glucose uptake	
		+	fasting insulin	
Non-diabetic Mexican Americans				[15]
	FABP2	+	2-h post challenge insulin levels	
Pondicherian Tamil Indians				[16]
	A54T	-	diabetes, body mass index,	
		-	waist/hip ratio, insulinemia	
		-	glycemia, triglyceride, total cholesterol	
French families with morbid obesity				[17]
	FABP2	-	body mass index, adult life body with gain fasting leptin, insulin, glycerol, free fatty acid	
B. Linkage study				
Mexican American diabetic pedigrees				[18]
	FABP2	-	diabetes	
French diabetic pedigrees				[19]
	FABP2	-	diabetes	
Pedigrees with lipodystrophic diabetes				[20]
	FABP2	-	lipodystrophic diabetes	
French families with morbid obesity				[17]
	FABP2	-	obesity	
C. Population association study				
UK, Finnish and Welsh population				[21]
	FABP2	-	diabetes/impaired glucose tolerance	
Pima Indian population				[9]
	A54T	-	diabetes	
Japanese population				[22]
	FABP2	-	diabetes/impaired glucose tolerance	
Aboriginal Canadians population				[28]
	A54T	-	diabetes	
Canadian Inuit population				[26]
	A54T	-	coronary artery disease	
Diabetic and nondiabetic Finnish population				[27]
	A54T	-	coronary artery disease	
Japanese population				[23]
	A54T	-	diabetes	
Japanese population				[24]
	A54T	-	diabetes, obesity, hypertension	
African Americans population				[25]
	A54T	-	diabetes	
D. Quantitative association study				
Non-diabetic Pima Indians				[9]
	A54T	+	fasting insulin, fasting fat oxidation	
		+	glucose uptake	

Table 4: Published studies of FABP2 and A54T

Aboriginal Canadians				[13]
	A54T	+	body mass index, percent body fat	
		+	fasting plasma triglyceride	
Finnish non-diabetic and diabetic subjects				[31]
	A54T	-	insulin sensitivity	
Obese Finnish subjects				[32]
	A54T	-	fatty acid composition	
Japanese subjects without fasting hyperglycemia				[14]
	A54T	+	2-hour post challenge insulin,	
		+	intraabdominal fat	
Aboriginal Canadians				[36]
	A54T	+	plasma triglyceride	
Finnish patients with familial combined hyperlipidemia				[29]
	A54T	+	lipid oxidation rate, HDL triglycerides,	
		+	LDL triglyceride	
		-	insulin sensitivity	
Obese Finnish subjects				[33]
	A54T	-	fasting insulin, glucose, lipid	
		-	lipoprotein, basal metabolic rate,	
		-	glucose and lipid oxidation	
Japanese population				[23]
	A54T	-	insulin sensitivity	
Japanese population				[24]
	A54T	-	dyslipidemia, hyperuricemia	
		-	hyperinsulinemia	
Guadeloupe Indian population				[30]
	A54T	+	triglyceride	
American Caucasian population				**
	A54T	+	insulin sensitivity (%S)	
E. Interventional study				
Response to dietary fiber in Canadian subjects				[36]
	A54T	+	greater decreases in total	
		+	LDL cholesterol and Apo-B	
Postprandial lipemic response in normotriglyceridemic subjects				[35]
	A54T	+	greater increases in chylomicron and VLDL triglycerides!	

* "+" denotes positive association or linkage and "-" denotes negative association or linkage; **the present study

Subjects and Methods

Subjects

Through the advertisement in the campus newspaper of this institution, healthy subjects without a prior history of diabetes and hypertension were invited to participate in the study. None of the participants were receiving medical treatment on a regular basis. Subjects were instructed to fast for at least 14 hours before the study visit. On the morning of the visit, subjects were admitted to the General Clinical Research Center of this institution as outpatients. An indwelling angiocatheter was inserted into an antecubital vein. All subjects had fasting blood samples drawn at -15, -10, and -5 minutes. A blood sample for the fasting lipid profile was obtained at -15 minutes. Fasting plasma glucose and insulin concentrations were calculated as the average of the three fasting samples. After an oral administration of 75-gm glucose, postchallenged blood samples were drawn at 30, 60, 90, and 120 minutes for glucose and insulin measurements. A total of 55 Caucasian subjects were enrolled in the study. They were glucose tolerant (fasting plasma glucose < 6.1 mM, interval plasma glucose < 11.1 mM, and 2-hour plasma glucose < 7.8 mM), and normotensive (systolic blood pressure < 140 mmHg and diastolic blood pressure < 90 mmHg). Plasma glucose and insulin concentrations were assayed as described previously [37]. Insulin sensitivity (%S) and beta-cell function (%B) were estimated based on the Homeostasis Model Assessment (HOMA) as described elsewhere [38,39]. They were calculated from the average of three fasting plasma glucose and insulin concentrations (mM and mU/L, respectively) using the following formulae: %S = 22.5 / (insulin x

glucose) and %B = 20 x insulin / (glucose - 3.5). The study was approved by the Institutional Review Board and written informed consents were obtained at the entry of the study from each participant. We confirm that the study has complied with the recommendations of the Declaration of Helsinki.

Genotyping

Genomic DNA was extracted from peripheral leukocytes using the method described previously [40]. A polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay was developed for genotyping. The genomic DNA fragment flanking the A54T polymorphism was amplified using two primers flanking exon 2 of the FABP2 gene: CTACCGAGTTTTCTTCCCACC and AATTAAACCATCCAATGAAATAGAGC. Polymerase chain reaction (PCR) was carried out in an 11-μl reaction volume containing 0.5 pM of each primer, 0.2 mM dNTP, 2 mM $MgCl_2$, 5% glycerol, 0.275 U Taq polymerase, 50 mM KC1, 10 mM Tris-HCl pH 8.3, and 0.1 μg of genomic DNA. The region of interest was amplified by an initial denaturation at 94°C for 5 minutes, 35 cycles of denaturation at 94°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 30 seconds, and concluded with a final extension at 72°C for 10 minutes. Then, 5 μl of the PCR product (375 base pairs) was digested in a 15-μl reaction volume containing 1 U of Hha I (New England Biolabs Inc., Beverly, Massachusetts, USA) with the buffer supplied by the vender. The digested PCR products were resolved on 2.0% agarose gels. Hha I digested the wild type, Alanine (GCT), which yielded two products, 200 and 175 base pairs (A allele). The G to A substitution (Threonine, ACT) destroyed the Hha I site (T allele).

Statistical analysis:

Variables, which failed the Normality test, were logarithmically transformed before analysis. They were age, body mass index, waist-hip ratio, plasma insulin concentrations, %S, and %B. The relationship between the variables and parameters of interest (%B or %S) was determined by using univariate analysis. A multivariate analysis using a stepwise-regression strategy was employed to examine the effect of covariates on the parameter of interest (%S and %B). The continuous covariates were age, body mass index, waist-hip ratio, and systolic and diastolic blood pressure. The categorical covariates were gender and the FABP2 polymorphism. Backward stepwise option with alpha-to-enter of 0.10 and alpha-to-remove of 0.10 was employed to exclude covariates that had much less or no influence on the parameter under analysis, one at a time starting from the one had least impact, which was based on the p value (the highest p value). Stepwise regression analysis was stopped when all the p values of all covariates, that were examined, were less than 0.10. Since a very close linear relationship was noted between systolic and diastolic pressure (r^2 = 0.3586, $p < 0.0001$) and also between body mass index and waist-hip ratio (r^2 = 0.3014, $p < 0.0001$), they were removed from the multivariate analysis based on their p values as indicated in each analysis. A nominal P value of less than 0.05 was considered significant. SYSTAT 8.0 for Windows from SPSS, Inc. (Chicago, Illinois) was used for the statistical analysis.

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