Brief Genetics Report

The Fatty Acid–Binding Protein-2 A54T Polymorphism Is Associated With Renal Disease in Patients With Type 2 Diabetes

Luis H. Canani,[1] Clarissa Capp,[1,2] Daniel P.K. Ng,[3,4,5] Serena G.L. Choo,[4] Ana Luiza Maia,[1] Gustavo B. Nabinger,[1] Kátia Santos,[6] Daisy Crispim,[6] Israel Roisemberg,[6] Andrzej S. Krolewski,[5] and Jorge L. Gross[1]

The intestinal fatty–acid binding protein-2 (*FABP2*) gene codes a protein responsible for the absorption of long-chain fatty acids. To test whether *FABP2* is a candidate gene for renal disease in patients with type 2 diabetes, a functional A54T polymorphism was genotyped in 1,042 Brazilians with type 2 diabetes. Patients were classified as having normoalbuminuria (urinary albumin excretion [UAE] <20 μg/min; *n* = 529), microalbuminuria (UAE 20–199 μg/min; *n* = 217), or proteinuria (UAE >199 μg/min; *n* = 160). Patients with end-stage renal disease (ESRD) (*n* = 136) were also included. The prevalence of the TT genotype was higher in patients with renal involvement compared with those with normoalbuminuria (odds ratio [95% CI] 2.4 [1.1–5.4]) following adjustment for type 2 diabetes duration, BMI, hypertension, A1C, and cholesterol levels. The risk was similar considering different stages of renal involvement. In a second independent patient sample (483 type 2 diabetic Caucasians residing in Massachusetts), a significant association was also observed between the TT genotype and proteinuria or ESRD (2.7 [1.0–7.3]; *P* = 0.048). This study thus provides evidence that *FABP2* confers susceptibility to renal disease in type 2 diabetic patients. *Diabetes* 54:3326–3330, 2005

From the [1]Endocrine Division, Hospital de Clínicas de Porto Alegre, Federal University of Rio Grande do Sul, Porto Alegre, Brazil; the [2]Department of Physiology, Federal University of Rio Grande do Sul, Porto Alegre, Brazil; the [3]Department of Community, Occupational and Family Medicine, National University of Singapore, Singapore; the [4]Centre for Molecular Epidemiology, National University of Singapore, Singapore; the [5]Section on Genetics and Epidemiology, Joslin Diabetes Center, Boston, Massachusetts; and the [6]Department of Genetics, Federal University of Rio Grande do Sul, Porto Alegre, Brazil.

Address correspondence and reprint requests to Jorge Luiz Gross, Serviço de Endocrinologia, Hospital de Clínicas de Porto Alegre, RS, Brazil, Rua Ramiro Barcelos 2350-Prédio 12-4° andar 90035-003 Porto Alegre, RS–Brazil. E-mail: jorgegross@terra.com.br.

Received for publication 17 January 2005 and accepted in revised form 22 July 2005.

Additional information for this article can be found in an online appendix at http://diabetes.diabetesjournals.org.

ESRD, end-stage renal disease; FABP2, fatty acid–binding protein-2; IHD, ischemic heart disease; SNP, single nucleotide polymorphism; UAE, urinary albumin excretion.

Diabetic nephropathy develops in up to 40% of diabetic patients at most, even when high glucose levels are maintained for long periods of time (1). Epidemiological (2) and familial (3–5) studies have demonstrated that genetic susceptibility contributes to the development of diabetic nephropathy in both type 1 and type 2 diabetes. The precise genetic model underlying diabetic nephropathy susceptibility is uncertain, but several genes with a minor effect have been associated with diabetic nephropathy (6). Insulin resistance has been implicated in the development of diabetic nephropathy (7), and aggregation of components of the metabolic syndrome is associated with a high prevalence of diabetic nephropathy (8). The intestinal fatty acid–binding protein-2 (*FABP2*) gene codes a protein expressed in enterocytes and is responsible for the absorption of long-chain fatty acids (9). A single nucleotide polymorphism (SNP) in the *FABP2* gene at codon 54 causes an amino acid change (Ala → Thr). This change affects the ability of the protein to bind and transport dietary fatty acids (9). Serum saturated fatty acids might induce endothelial dysfunction (10) and are related to increased cardiovascular mortality (11). We have previously reported increased levels of serum saturated fatty acids in patients with type 2 diabetes and microalbuminuria (12), as well as a reduction in albumin excretion rate after replacement of red meat (high content of saturated fatty acids) with chicken (low content of saturated fatty acids) in these patients (13). Based on these observations, we hypothesized that genetically predisposed subjects exposed to a diet with a high content of saturated fatty acids might be at high risk for developing renal disease. Therefore, we decided to examine whether *FABP2* is a susceptibility gene for renal disease in patients with type 2 diabetes. Our study focused particularly on the A54T polymorphism, since this polymorphism results in a functionally altered FABP2 protein.

RESEARCH DESIGN AND METHODS

A total of 1,042 patients with type 2 diabetes were identified from a multicentric study that started recruiting patients in southern Brazil in 2002. That project aimed to study risk factors for chronic complications of diabetes. It included four centers located at general hospitals in the state of Rio Grande do Sul, namely Grupo Hospitalar Nossa Senhora da Conceição, Hospital São Vicente de Paula, Hospital Universitário de Rio Grande, and Hospital de Clínicas de Porto Alegre. All patients with type 2 diabetes attending the

endocrine clinics in these hospitals, as well as the patients receiving dialysis in these institutions, were included. Type 2 diabetes was defined by diagnosis of diabetes after the age of 35 years with no use of insulin during the 1st year after diagnosis. Patients' renal status was categorized according to urinary albumin excretion (UAE; three samples, 6 months apart, without ACE inhibitors or angiotensin type I receptor blockers for at least 1 week) into normoalbuminuria ($n = 529$), microalbuminuria ($n = 217$), proteinuria ($n = 160$) (14), or the presence of end-stage renal disease (ESRD; $n = 136$).

Patients underwent a standardized evaluation consisting of a questionnaire, physical examination, and laboratory tests. Weight without shoes and in light outdoor clothes and height were measured, and BMI was calculated. Measurements of waist circumference at the narrowest point as viewed from the front and hip at the widest point were performed, and waist-to-hip ratio was calculated. Hypertension was defined as blood pressure ≥140/90 mmHg or use of antihypertensive medication. An ophthalmologist performed direct fundoscopy, and the presence of diabetic retinopathy was classified as absent, nonproliferative, or proliferative. Ischemic heart disease (IHD) was diagnosed as previously described (8). Briefly, IHD was established in the presence of angina or possible infarct according to the World Health Organization Cardiovascular Questionnaire and/or the presence of resting electrocardiogram abnormalities (Minnesota Code Q and QS patterns [1.1–2, 1.3]; S-T junction [J] and segment depression [4.1–4]; T wave items [5.1–3], and complete left bundle block [7.1]) and/or the presence of perfusion abnormalities (fixed or variable) upon myocardial scintigraphy at rest and after dipyridamole administration. Because the diagnosis of IHD also requires scintigraphy, it was assessed in only 685 patients. The local ethics committees approved the protocol, and all patients signed an informed consent form.

The possible association between A54T and renal involvement was also tested in an independent patient sample consisting of Caucasians with type 2 diabetes residing in Massachusetts. The characteristics of this patient collection have already been previously described in detail (15). Briefly, this is a case-control study where case subjects were patients with type 2 diabetes and advanced renal disease as indicated by the presence of persistent proteinuria or impaired renal function, whereas control subjects were normoalbuminuric despite having type 2 diabetes for >6 years. At the time of this study, DNA was available for 201 control subjects and 282 case subjects; 41% of case subjects had impaired renal function. The Committee on Human Subjects of the Joslin Diabetes Center approved the protocols and informed consent procedures for this study.

Laboratory analysis. UAE was measured by immunoturbidimetry (Sera-Pak immunomicroalbuminuria; Bayer, Tarrytown, NY; intra- and interassay coefficients of variation: 4.5 and 11.0%, respectively) (14). UAE was measured using two collection methods: a 24-h timed urine collection ($n = 691$) or random spot ($n = 215$) sterile urine samples. The cutoff values used to define the stages of renal involvement followed American Diabetes Association recommendations for timed urine collections (μg/min). For spot samples, urine concentration (mg/l) cutoffs were used as previously validated in our central laboratory (14) into normoalbuminuria (UAE <20 μg/min or <17 mg/l), microalbuminuria (UAE 20–199 μg/min or 17–174 mg/l), and proteinuria (UAE ≥200 μg/min or >174 mg/l). Glucose was determined by a glucose oxidase method, creatinine by the Jaffé reaction, A1C test by an ion-exchange high-performance liquid chromatography procedure (Merck-Hitachi L-9100 glycated hemoglobin analyzer, reference range 2.7–4.3%; Merck, Darmstadt, Germany), and triglyceride and cholesterol levels by enzymatic methods.

Molecular analysis. DNA was isolated from lymphocytes using standard procedures (16). Subjects were genotyped for *FABP2* A54T polymorphism as previously described (12). All PCRs were run in a final volume of 25 μl, containing 50 ng of genomic DNA, 20 mmol/l Tris-Cl, (pH 8.4), 50 mmol/l KCl, 1.5 mmol/l $MgCl_2$, 0.2 mmol/l dNTPs, 1 unit of *Taq*DNA polymerase, and 1 μmol/l of specific primer.

To define the linkage disequilibrium between the A54T polymorphism and other polymorphisms located in *FABP2*, the International HapMap Project website (www.hapmap.org) was consulted. There were three SNPs genotyped in the Caucasian samples that were located within the *FABP2* locus, not including our SNP. To determine linkage disequilibrium among these SNPs, we genotyped the 90 subjects from the HapMap sample for A54T polymorphisms. A Haploview file was created to evaluate linkage disequilibrium among the polymorphisms (95% CI), as described by Gabriel et al. (17).

Statistical analysis. Continuous data were expressed as means ± SD. Categorical data were expressed as number of case subjects and percent of individuals affected. χ^2, Student's t test, or one-way ANOVA were used to compare genotype groups in terms of clinical and laboratory characteristics. Tukey's test was used for post hoc multiple comparisons. Hardy-Weinberg equilibrium was calculated using allele frequencies and the χ^2 test. To examine the main effect of the *FABP2* A54T variant, the three genotypes (AA, AT, and TT) were considered separately, followed by pooling of the genotypes (AT/AA vs. TT or AA vs. AT/TT) to evaluate the model of inheritance (dominant or recessive). The magnitude of the association was estimated using odds ratios (ORs) with 95% CIs. Variables without normal distribution were log transformed. To estimate the adjusted OR, multiple logistic regression analysis was performed with renal involvement as the dependent variable. $P < 0.05$ (two sided) was considered to be significant.

RESULTS

Brazilian sample. Brazilian patients with type 2 diabetes and some degree of renal involvement were more often males, had longer type 2 diabetes duration, and had higher systolic blood pressure levels (Table 1). Increased prevalence of diabetic retinopathy corresponded to renal status ($P < 0.001$). Patients with proteinuria and ESRD had a higher prevalence of IHD than normo- or microalbuminuric patients ($P = 0.001$). Patients with ESRD had lower BMI and total cholesterol than the other patients. Patients with proteinuria and ESRD had lower HDL cholesterol, and proteinuric patients had higher triglyceride levels than normoalbuminuric patients. Metabolic control was similar in all groups.

The distribution of the A54T genotypes is presented in Table 1. The frequency of the T allele (risk allele) was 0.25, and the genotypes were in Hardy-Weinberg equilibrium. Patients with renal involvement (microalbuminuria, proteinuria, or ESRD) had increased frequency of the TT genotype (10.1%) compared with normoalbuminuric patients (4.5%; $P = 0.002$). Assuming a dominant model (AA vs. AT/TT), the presence of the T allele was not associated with the presence of renal involvement (OR [95% CI] 1.2 [0.93–1.5]; $P = 0.162$). However, considering a recessive model, the TT genotype was associated with the presence of renal disease (AA/AT vs. TT, 2.4 [1.4–4.0]; $P = 0.0005$). In the recessive model, the OR adjusted for type 2 diabetes duration, BMI, hypertension, A1C, and cholesterol levels was similar to the crude estimate (adjusted OR 2.4 [1.1–5.4]), suggesting that the TT genotype is independently associated with renal disease in patients with type 2 diabetes.

Assuming the recessive model (AA/AT vs. TT), we evaluated the effect of the polymorphism on the different stages of renal involvement. The adjusted ORs (type 2 diabetes duration, BMI, hypertension, A1C, and cholesterol) are presented in Fig. 1.

Patients with the TT ($n = 76$) genotype were similar to patients with AA ($n = 553$) and AT ($n = 413$) genotypes regarding the proportion of males (54, 42, and 45%, respectively, $P = 0.105$), ethnicity (Caucasians 80, 75, and 80%, respectively, $P = 0.718$), mean age (60 ± 10, 59 ± 10, and 58 ± 11 years, respectively, $P = 0.250$), known type 2 diabetes duration (12 ± 8, 12 ± 1, and 12 ± 1 years, respectively, $P = 0.837$), BMI (29.3 ± 5.4, 28.7 ± 5.2, and 28.9 ± 5.1 kg/m^2, respectively, $P = 0.543$), waist-to-hip ratio (0.95 ± 0.08, 0.94 ± 0.08, and 0.95 ± 0.08, respectively, $P = 0.157$), blood pressure levels (systolic 139 ± 21, 143 ± 24, and 143 ± 23 mmHg, respectively, $P = 0.444$; diastolic 85 ± 13, 86 ± 12, and 87 ± 15 mmHg, respectively, $P = 0.414$), and prevalence of hypertension (68, 67, and 68%, respectively, $P = 0.852$). Glycemic control (A1C 7.12 ± 1.98, 6.63 ± 1.90, and 6.76 ± 2.08, respectively, $P = 0.145$; fasting plasma glucose 200 ± 87, 178 ± 75, and 180 ± 71 mg/dl, respectively, $P = 0.196$) and lipid profile (total cholesterol 206 ± 48, 215 ± 47, and 220 ± 51 mg/dl, respectively, $P = 0.218$; HDL cholesterol 43 ± 17, 44 ± 12, and 44 ± 11 mg/dl, respectively, $P = 0.828$; triglycerides OR [95% CI] 168 [34–999], 149 [29–1,470], and 156 [43–1,439] mg/dl, respectively, $P = 0.266$) were also similar in

TABLE 1
Characteristics of Brazilian type 2 diabetic patients according to renal status

	Normoalbuminuria	Microalbuminuria	Proteinuria	ESRD	*P**
n	529	217	160	136	
Male	190 (35.9)	106 (48.8)	91 (56.9)	80 (58.8)	<0.001†
Caucasian	407 (76.9)	159 (73.3)	117 (73.1)	97 (71.3)	0.438
Age (years)	58.3 ± 10	58.9 ± 11	60.1 ± 10	60.2 ± 11	0.218
Duration of diabetes (years)	9.8 ± 7.6	13.1 ± 9.8	15.1 ± 8.6	16.2 ± 8.2	<0.001†‡
BMI (kg/m^2)	28.7 ± 5.45	28.8 ± 4.84	30.1 ± 5.4	27.0 ± 4.17	<0.001§
Waist-to-hip ratio	0.94 ± 0.07	0.95 ± 0.08	0.97 ± 0.08	0.92 ± 0.12	0.061
Systolic blood pressure (mmHg)	139 ± 23	144 ± 24	151 ± 25	150 ± 22	<0.001†
Diastolic blood pressure (mmHg)	86 ± 13	88 ± 13	88 ± 16	83 ± 11	0.006§
Hypertension	312 (59)	162 (75)	134 (84)	116 (85)	<0.001†‡
Retinopathy (Absent/NPDR/PDR [%])	70/24/06	43/40/17	20/41/39	15/06/79	<0.001†‡
IHD¶	123 (33.6)	55 (39.3)	57 (50.4)	36 (54.5)	0.001
A1C (%)	6.67 ± 1.92	6.78 ± 2.04	6.67 ± 2.22	6.79 ± 1.96	0.736
Fasting plasma glucose (mg/dl)	177 ± 69	193 ± 97	181 ± 83	169 ± 83	0.453
Serum creatinine (mg/dl)	0.91 (0.5–1.3)	0.94 (0.4–1.2)	1.3 (0.6–5.0)	5.85 (2–18)	—
Total cholesterol (mg/dl)	215 ± 46	218 ± 47	227 ± 53	194 ± 46	<0.001§
HDL cholesterol (mg/dl)	45 ± 12	43 ± 12	42 ± 12	42 ± 13	0.011‖
Triglycerides (mg/dl)	144 (26–946)	150 (43–1,470)	192 (47–1,265)	181 (59–900)	<0.001#
A54T *FABP2* genotypes (AA/AT/TT)	292/213/24	113/85/19	78/68/14	70/47/19	0.008†

Data are means ± SD, median (range), or *n* (%) of cases. *ANOVA, χ^2, or Kruksal-Wallis test; †normoalbuminuria vs. all; ‡microalbuminuria vs. all; §ESRD vs. all; ¶data available for 685 patients: normoalbuminuria, *n* = 366; microalbuminuria, *n* = 140; proteinurics, *n* = 113; and ESRD, *n* = 66; ‖normoalbuminuria vs. proteinuria and ESRD; #proteinuria vs. all. NPDR, nonproliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy.

the three genotypes. When patients with serum creatinine <1.5 mg/dl were analyzed to avoid interference of the renal status on the lipid profile, the triglyceride levels were higher in those with the TT genotype (171 [34–899] mg/dl) than those with the AA genotype (147 [27–600], $P = 0.019$), but similar to those with the AT genotype (162 [37–877] mg/dl). The group with the TT genotype also had lower total cholesterol compared with the group with the AA genotype (191 ± 50 vs. 217 ± 44 mg/dl, $P = 0.026$). The levels of total cholesterol in the AT group (220 ± 42 mg/dl) were not different from the AA or TT groups. None of the other clinical and laboratory parameters differed across the genotypes in this subset of patients (data not shown).

The frequency of the TT genotype was the same for patients with normoalbuminuria and known duration of type 2 diabetes >5 years (4.7%) or >10 years (4.8%), indicating that type 2 diabetes duration >5 years did not cause further depletion of the TT genotype among normoalbuminuric patients. Considering only white Brazilians, the TT genotype distribution was similar to the entire group (4.7% normoalbuminuric, 10.8% renal disease).

Joslin sample. To test the a priori hypothesis that the TT genotype confers risk for diabetic nephropathy in other diabetic populations, we genotyped this marker in an independent case-control study of 483 Caucasians with type 2 diabetes residing in the state of Massachusetts. Patients with microalbuminuria were not included in the U.S. collection (15). The frequency of the T allele among normoalbuminuric patients was similar in the American and Brazilian samples (0.20 vs. 0.25, $P = 0.086$). In 201 control subjects with type 2 diabetes and normoalbuminuria, the frequency of genotypes was 61.7% (124) for AA, 35.8% (72) for AT, and 2.5% (5) for TT. In 282 case subjects with type 2 diabetes and overt proteinuria or ESRD, the frequency of genotypes was 57.1% (161) for AA, 36.5% (103) for AT, and 6.4% (18) for TT. As with the Brazilian dataset, the TT genotype was significantly associated with diabetic nephropathy in the U.S. dataset (one-tailed χ^2 test,

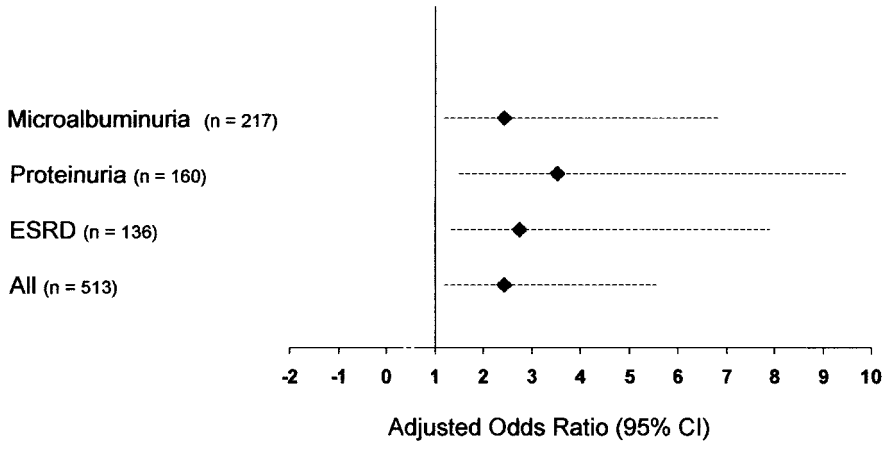

FIG. 1. OR (♦) with 95% CI (dotted line) for the presence of *FABP2* A54T polymorphism in the different stages of renal involvement adjusted for duration of diabetes, BMI, hypertension, A1C, and cholesterol levels.

$P = 0.024$). Employing the more stringent two-tailed χ^2 test, the association between TT and diabetic nephropathy remained statistically significant ($P = 0.048$), with an OR of 2.7 (95% CI 1.0 –7.3).

Thus, concurring with the results of the Brazilian study, the *FABP2* polymorphism was significantly associated with proteinuria and ESRD, with the TT genotype conferring greater risk of disease compared with genotypes bearing the A allele.

Haplotype analyses. We considered the possibility that there may be several potential haplotypes that harbor the 54T allele, of which there may be only one that is directly associated with risk for renal disease. To gain insight into this possibility, we analyzed International HapMap Project data for the *FABP2* locus. These data included Caucasian genotypes for 22 SNPs (Table 1, online appendix [available at http://diabetes.diabetesjournals.org]) spanning 50 kb from position 120693944 (rs10518301) to 120743790 (rs11098506) that contain the 4.9-kb *FABP2* locus. Considering only the haplotypes with a frequency >2%, we identified six haplotypes that capture 91.6% of the variability in this 50-kb region (Table 2, online appendix). Notably, 54T was present in only one haplotype. This is consistent with the hypothesis that the observed gene-disease association is related to the presence of this 54T allele or the one specific haplotype that bears it.

DISCUSSION

In these two samples of patients with type 2 diabetes, the presence of the A54T polymorphism of the *FABP2* gene was associated with a two- to threefold increased risk for renal involvement. The magnitude of the association was the same for microalbuminuria, proteinuria, and/or ESRD, suggesting that this DNA variant is associated with the development of renal disease beginning in its early stages. To our knowledge, this is the first time this association has been reported.

The A54T polymorphism of the *FABP2* gene has been associated with an increase in FABP2 affinity for long-chain fatty acids such as stearic and palmitic acids and consequently with higher levels of these fatty acids after a test meal (18). We have previously reported that patients with type 2 diabetes and microalbuminuria had higher levels of saturated fatty acids, especially stearic and palmitic acids, than normoalbuminuric patients (12). Saturated fatty acids might be related to endothelial dysfunction (10), and there is epidemiological evidence that they are risk factors for cardiovascular mortality (11). The possible mechanism through which the A54T polymorphism causes renal disease has not been established but might be related to endothelial dysfunction and glomerular damage due to higher levels of saturated fatty acids. Moreover, this polymorphism has also been associated with insulin resistance (19–21) and higher levels of triglycerides in the fasting state and after a fatty meal challenge (18,22). In the present study, the association of the TT genotype with elevated triglycerides was only evident among patients without renal function impairment (serum creatinine <1.5 mg/dl). In the total group, this effect was not seen probably due to interference from the renal failure or dietary orientation. None of these patients were on lipid-lowering drugs at the time of the evaluation.

We should be cautious regarding the results of positive associations in cross-sectional studies. Many of the associations reported are spurious and not confirmed by replication studies. To minimize such effect, it has been proposed that genetic association studies should include a large number of patients, low *P* values, and a priori clear biological hypothesis (23). In the present study, the a priori hypothesis was based on our previous observations; the sample was large enough to detect an OR of 2.4 with a power of 92% and to reach a low *P* value ($P = 0.0005$). To assess whether these results could be replicated, a sample of American patients with type 2 diabetes was evaluated. In this independent group, the same pattern and magnitude of association was observed. Furthermore, the frequency of the T allele observed in the Brazilian sample was similar to that observed in other diabetic populations (Pima Indians, white Europeans) but lower than described for Guadeloupe Indians or Asians (9).

The linkage disequilibrium map showed that the A54T polymorphism is located in a haploblock that spans 50 kb and includes 22 SNPs. However, among the frequent haplotypes (>2%), the T54 allele is present in only one of six possibilities. It is interesting that in this haploblock, there are no other known or putative genes except for *FABP2*. At present, we cannot exclude that one of the other DNA sequence differences on this haplotype but within the *FABF2* locus could be causally related to diabetic kidney disease. However, this would require sequencing this very long haplotype together with functional studies, a task that is beyond the scope of this study.

Another aspect that needs to be considered in association studies is survival bias. In the scenario of an important survival effect, the increased prevalence of the TT genotype among case subjects would reflect that this polymorphism is associated with increased survival. Since this is a cross-sectional study, we cannot completely rule out this possibility. However, there is evidence suggesting that this was not the case. Based on the putative mechanism of the polymorphism, one concern was the association with IHD. These data were available for only a subset of the patients, and no association was demonstrated. Furthermore, if survival were an important contribution to our findings, we would expect that the TT frequency would change according to type 2 diabetes duration. This was not observed.

In conclusion, the A54T *FABP2* polymorphism is associated with renal involvement in type 2 diabetic patients even in early stages such as microalbuminuria. However, this gene seems to have a minor effect, since the frequency of the risk genotype among case subjects was only ~10%. Therefore, the search for other genes involved in the genetic predisposition for this diabetes complication is needed.

ACKNOWLEDGMENTS

This study was partially supported by grants from Projeto de Núcleos de Excelência do Ministério de Ciência e Tecnologia and Hospital de Clínicas de Porto Alegre. Additional support was provided by grant DK-058549 from the National Institutes of Health and grant NMRC/0,850/2004 from the National Medical Research Council, Singapore. L.H.C. is the recipient of a postdoctoral grant, and C.C. is the recipient of a scholarship from the Fundação de Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior (Fundação CAPES).

REFERENCES

1. Wong TY, Shankar A, Klein R, Klein BE: Retinal vessel diameters and the incidence of gross proteinuria and renal insufficiency in people with type 1 diabetes. *Diabetes* 53:179–184, 2004
2. Krolewski AS, Warram JH, Christlieb AR, Busick EJ, Kahn CR: The changing natural history of nephropathy in type I diabetes. *Am J Med* 78:785–794, 1985
3. Quinn M, Angelico MC, Warram JH, Krolewski AS: Familial factors determine the development of diabetic nephropathy in patients with IDDM. *Diabetologia* 39:940–945, 1996
4. Canani LH, Gerchman F, Gross JL: Familial clustering of diabetic nephropathy in Brazilian type 2 diabetic patients. *Diabetes* 48:909–913, 1999
5. Seaquist ER, Goetz FC, Rich S, Barbosa J: Familial clustering of diabetic kidney disease: evidence for genetic susceptibility to diabetic nephropathy. *N Engl J Med* 320:1161–1165, 1989
6. Krolewski AS, Ng DP, Canani LH, Warram JH: Genetics of diabetic nephropathy: how far are we from finding susceptibility genes? *Adv Nephrol Necker Hosp* 31:295–315, 2001
7. Yip J, Mattock MB, Morocutti A, Sethi M, Trevisan R, Viberti G: Insulin resistance in insulin-dependent diabetic patients with microalbuminuria. *Lancet* 342:883–887, 1993
8. Costa LA, Canani LH, Lisboa HR, Tres GS, Gross JL: Aggregation of features of the metabolic syndrome is associated with increased prevalence of chronic complications in type 2 diabetes. *Diabet Med* 21:252–255, 2004
9. Weiss EP, Brown MD, Shuldiner AR, Hagberg JM: Fatty acid binding protein-2 gene variants and insulin resistance: gene and gene-environment interaction effects. *Physiol Genomics* 10:145–157, 2002
10. Steer P, Vessby B, Lind L: Endothelial vasodilatory function is related to the proportions of saturated fatty acids and alpha-linolenic acid in young men, but not in women. *Eur J Clin Invest* 33:390–396, 2003
11. Ohrvall M, Berglund L, Salminen I, Lithell H, Aro A, Vessby B: The serum cholesterol ester fatty acid composition but not the serum concentration of alpha tocopherol predicts the development of myocardial infarction in 50-year-old men: 19 years follow-up. *Atherosclerosis* 127:65–71, 1996
12. Perassolo MS, Almeida JC, Pra RL, Mello VD, Maia AL, Moulin CC, Camargo JL, Zelmanovitz T, Azevedo MJ, Gross JL: Fatty acid composition of serum lipid fractions in type 2 diabetic patients with microalbuminuria. *Diabetes Care* 26:613–618, 2003
13. Gross JL, Zelmanovitz T, Moulin CC, De Mello V, Perassolo M, Leitao C, Hoefel A, Paggi A, Azevedo MJ: Effect of a chicken-based diet on renal function and lipid profile in patients with type 2 diabetes: a randomized crossover trial. *Diabetes Care* 25:645–651, 2002
14. Zelmanovitz T, Gross JL, Oliveira JR, Paggi A, Tatsch M, Azevedo MJ: The receiver operating characteristics curve in the evaluation of a random urine specimen as a screening test for diabetic nephropathy. *Diabetes Care* 20:516–519, 1997
15. Ng DP, Walker WH, Chia KS, Choo S, Warram JH, Krolewski AS: Scrutiny of the glutamine-fructose-6-phosphate transaminase 1 (GFPT1) locus reveals conserved haplotype block structure not associated with diabetic nephropathy. *Diabetes* 53:865–869, 2004
16. Sambrock J, Fritsch EF, Mariatis T: *Molecular Cloning: A Manual Laboratory*. Cold Spring Harbor, NY, Cold Spring Harbor Laboratory, 1990
17. Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, DeFelice M, Lochner A, Faggart M, Liu-Cordero SN, Rotimi C, Adeyemo A, Cooper R, Ward R, Lander ES, Daly MJ, Altshuler D: The structure of haplotype blocks in the human genome. *Science* 296:2225–2229, 2002
18. Agren JJ, Vidgren HM, Valve RS, Laakso M, Uusitupa MI: Postprandial responses of individual fatty acids in subjects homozygous for the threonine- or alanine-encoding allele in codon 54 of the intestinal fatty acid binding protein 2 gene. *Am J Clin Nutr* 73:31–35, 2001
19. Albala C, Santos JL, Cifuentes M, Villarroel AC, Lera L, Liberman C, Angel B, Perez-Bravo F: Intestinal FABP2 A54T polymorphism: association with insulin resistance and obesity in women. *Obes Res* 12:340–345, 2004
20. Chiu KC, Chuang LM, Chu A, Yoon C: Fatty acid binding protein 2 and insulin resistance. *Eur J Clin Invest* 31:521–527, 2001
21. Galluzzi JR, Cupples LA, Meigs JB, Wilson PW, Schaefer EJ, Ordovas JM: Association of the Ala54-Thr polymorphism in the intestinal fatty acid-binding protein with 2-h postchallenge insulin levels in the Framingham Offspring Study. *Diabetes Care* 24:1161–1166, 2001
22. Berthier MT, Couillard C, Prud'homme D, Nadeau A, Bergeron J, Tremblay A, Despres JP, Vohl MC: Effects of the FABP2 A54T mutation on triglyceride metabolism of viscerally obese men. *Obes Res* 9:668–675, 2001
23. Freely associating (Editorial). *Nat Genet* 22:1–2, 1999