Brief Genetics Report

The Ala54Thr Polymorphism of the Fatty Acid Binding Protein 2 Gene Does Not Influence Insulin Sensitivity in Finnish Nondiabetic and NIDDM Subjects

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Insulin resistance and hyperinsulinemia are major predictors of NIDDM. Since several studies have demonstrated that heredity plays a significant role in the development of insulin resistance (1), defects in genes that regulate insulin action could potentially contribute to the risk of NIDDM. A locus on chromosome 4q has been shown to be linked with fasting insulin levels (2), 2-h insulin levels (3,4), and insulin action (2) in Pima Indians and Mexican-Americans, suggesting that the fatty acid binding protein 2 (FABP2) gene is a promising candidate gene for insulin resistance and NIDDM.

The FABP2 gene encodes the intestinal fatty acid binding protein that plays an important role in the absorption and intracellular transport of dietary long-chain fatty acids. An amino acid substitution Ala54Thr of the FABP2 gene has been reported to be associated with insulin resistance, measured by euglycemic hyperinsulinemic clamp in nondiabetic Pima Indians (5). However, no studies are available on this association in Caucasian subjects. Therefore, we screened the FABP2 gene for variants in Finnish diabetic and nondiabetic subjects and investigated the possible association of the common polymorphism in codon 54 of this gene with insulin resistance.

Altogether 110 patients with NIDDM (55 men and 55 women; age, 63 ± 1 years; BMI, 30.4 ± 0.5 kg/m^2) who participated in our previous population study were included (6). Control subjects were 82 healthy men (age, 54 ± 1 years; BMI, 27.2 ± 0.5 kg/m^2) who did not have any chronic diseases, abnormality in an oral glucose tolerance test, or hypertension (use of antihypertensive drugs or systolic/diastolic blood pressure >160/95 mmHg). None was receiving any drug treatment that could influence glucose metabolism.

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FABP2, fatty acid binding protein 2; PCR, polymerase chain reaction; SSCP, single-strand conformation polymorphism.

On the first day, the NIDDM and control subjects underwent an oral glucose tolerance test (75 g glucose) after 12-h fast. On a separate day, the degree of insulin resistance was evaluated with the euglycemic clamp technique and indirect calorimetry after 12-h fast, and the rates of glucose oxidative and nonoxidative disposal were assessed as previously described (7). The protocol was approved by the Ethics Committee of the University of Kuopio.

All four exons of the FABP2 gene were amplified with the polymerase chain reaction (PCR) using primers and PCR conditions essentially as they have been described previously (5). To obtain PCR products smaller than 200 base pairs (bp) for single-strand conformation polymorphism (SSCP) analysis, the PCR products of exons 2 and 3 were digested with restriction enzymes *Hin*fI (exon 2) and *Ava* II (exon 3), respectively. SSCP analysis was performed essentially according to the method of Orita et al. (8). Each sample was run at two different gel temperatures: *1*) at 38°C for ~4 h and *2*) at 29°C for ~5 h. Variants of the FABP2 gene from individuals with abnormally migrating bands in SSCP analysis were sequenced as previously described (7). To determine the Ala54Thr substitution, amplified segments of exon 2 were also digested with the *Hha* I enzyme. PCR products lacking the *Hha* I restriction site migrated as 274-bp fragments, whereas PCR products containing an intact *Hha* I site were cleaved into 145-bp and 129-bp fragments.

Initial screening of all four exons of the FABP2 gene included 53 diabetic and 40 nondiabetic subjects randomly selected from 110 NIDDM patients and 82 control subjects. Among these subjects, we found an amino acid polymorphism Ala54Thr (exon 2), a silent substitution GTA118GTG (exon 4), and a sequence variant GCGCA to GCACA in the 3′ noncoding region (not reported previously). The allelic frequencies of variants in control subjects and NIDDM patients, respectively, were as follows: the Ala54Thr polymorphism, 0.31 and 0.27 (NS); the silent substitution in codon 118, 0.34 and 0.33 (NS); and the sequence variation of 3′ noncoding region, 0.05 and 0.03 (NS).

We also found a 3-bp repeat sequence $(ATT)_n$ in intron 2 (6 nucleotides upstream from exon 3). The number of this repeat sequence varied between 10 and 15 in control subjects and between 10 and 14 in diabetic patients (NS). In the whole

TABLE 1
Plasma glucose, insulin, and serum free fatty acid levels and the results of metabolic studies in control subjects (n = 81) and NIDDM patients (n = 19) according to the codon 54 polymorphism of the FABP2 gene

	Control subjects		NIDDM patients	
	Ala54Ala	Thr54 allele	Ala54Ala	Thr54 allele
n	39	42	13	6
Fasting glucose (mmol/l)	5.7 ± 0.1	5.5 ± 0.1	9.3 ± 0.9	9.6 ± 1.3
Fasting insulin (pmol/l)	57.0 ± 6.3	55.2 ± 5.2	138.4 ± 20.9	180.0 ± 50.8
1-h insulin (pmol/l)	417.1 ± 63.0	440.3 ± 59.7	359.4 ± 85.4	457.3 ± 113.6
2-h insulin (pmol/l)	213.8 ± 29.6	207.6 ± 42.7	419.0 ± 110.5	450.7 ± 115.6
Fasting free fatty acids (mmol/l)	0.46 ± 0.03	0.49 ± 0.03	0.93 ± 0.07	0.81 ± 0.12
Whole body glucose uptake ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)	56.9 ± 2.3	59.0 ± 2.3	30.9 ± 3.0	25.3 ± 1.9
Glucose oxidation ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)	19.7 ± 0.8	19.2 ± 0.7	12.1 ± 1.3	10.7 ± 0.6
Glucose nonoxidation ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)	37.2 ± 2.0	39.8 ± 2.0	18.8 ± 2.2	14.6 ± 1.7
Lipid oxidation ($mg \cdot kg^{-1} \cdot min^{-1}$)	–0.03 ± 0.04	0.03 ± 0.03	0.37 ± 0.08	0.34 ± 0.09

Data are means ± SE. The subjects with the Thr 54 allele (Ala54Thr or Thr54Thr) were pooled. Insulin levels were log-transformed before statistical analysis. None of the comparisons (two-tailed Student's t test) between the groups (Ala54Ala and Thr54 allele) was statistically significant.

study population, the frequency of the Thr-encoding allele was 0.28 in the NIDDM group (n = 110) and 0.29 in the control group (n = 82) (NS). There were nine NIDDM patients and five control subjects who were homozygous for the Thr-encoding allele. Metabolic studies were available for 81 healthy control subjects and 19 NIDDM patients. Table 1 shows that glucose, insulin, and free fatty acid levels, glucose oxidative and glucose nonoxidative disposal, and lipid oxidation were not significantly influenced by the Ala54Thr polymorphism.

To summarize, we did not find variants in the FABP2 gene specific for patients with NIDDM. Furthermore, all variants, including a new sequence variant in the 3′ noncoding region, were found at a similar frequency in both the NIDDM patients and the control subjects. The Ala54Thr polymorphism was not associated with high fasting or postchallenge insulin levels or with insulin resistance assessed by euglycemic clamp. In fact, nondiabetic subjects with the Thr54 allele tended to be more insulin sensitive than subjects with the Ala54Ala genotype. Moreover, no effect of this polymorphism on lipid oxidation rate was found either in nondiabetic or diabetic subjects. These observations are in accordance with recent studies that indicate that the FABP2 gene is not a major genetic determinant of insulin resistance in Caucasians (9,10) or Mexican-Americans (11,12).

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