

Variants of the Fatty Acid-Binding Protein 2 Gene Are Not Associated With Coronary Heart Disease in Nondiabetic Subjects and in Patients With NIDDM

LAURA SAARINEN
ARTO PULKKINEN, MD
ANU KAREINEN, MD

SAMI HEIKKINEN, MSC
SEPPÖ LEHTO, MD
MARKKU LAAKSO, MD

OBJECTIVE— To investigate the association of variants of the fatty acid-binding protein (FABP) 2 gene with coronary heart disease (CHD) in nondiabetic subjects and in patients with NIDDM.

RESEARCH DESIGN AND METHODS— Cross-sectional study included 135 nondiabetic and 79 NIDDM subjects with stenosis (>50%) in at least two coronary arteries. A group of 81 healthy nondiabetic men without CHD served as a control population. All exons and intron-exon junctions of the FABP2 gene were amplified with the polymerase chain reaction, and variants were screened with the single-strand conformation polymorphism analysis.

RESULTS— The allele frequency of an amino acid polymorphism (alanine→threonine) in codon 54 of exon 2 of the FABP2 gene was 0.26 in nondiabetic subjects with CHD and 0.27 in NIDDM subjects with CHD. Other variants (GTA 118 GTC, GCGCA→GCACA in the 3'-noncoding region, and the number of ATT repeats in intron 2) also did not associate with CHD.

CONCLUSIONS— The variants of the FABP2 gene are not likely to contribute to the risk of CHD in Finnish nondiabetic and NIDDM subjects.

Coronary heart disease (CHD) clusters in families, indicating that hereditary factors play a significant role in the etiology of this disease (1,2). Gene defects leading to decreased insulin sensitivity might contribute to CHD because prospective population-based studies have indicated an association of hyperinsulinemia with CHD (3).

The fatty acid-binding protein 2 (FABP2) gene encodes an intestinal fatty acid-binding protein that is expressed only in the columnar absorptive epithelial cells of the small intestinal villus (4). Defects in the FABP2 gene could affect the binding capacity of the FABP2 protein, increase fatty acid absorption, and lead to enhanced fatty acid oxidation and an impairment in

insulin action. In fact, the polymorphism Ala→Thr in codon 54 of the FABP2 gene has been shown to be associated with insulin resistance in nondiabetic Pima Indians (5).

To investigate the possibility that defects in the FABP2 gene could contribute to the risk of CHD, we screened the entire coding region of the FABP2 gene in nondiabetic and NIDDM subjects with and without CHD.

RESEARCH DESIGN AND METHODS

Subjects

Nondiabetic subjects with CHD ($n = 135$; 93 men, 42 women; 54 ± 1 years of age,

BMI 26 ± 1 kg/m²) included in the study had chest pain symptoms and stenosis >50% (verified by coronary angiography) at least in two coronary arteries. Diabetic subjects ($n = 79$; 61 men, 18 women; 61 ± 1 years of age, BMI 29.0 ± 1 kg/m²) had NIDDM according to World Health Organization criteria (6), and they had chest pain symptoms and stenosis >50% in at least two coronary arteries. Nondiabetic subjects without CHD ($n = 81$) were healthy, unrelated men with normal oral glucose tolerance tests from our previous population-based study (7).

The protocol was approved by the Ethics Committee of the University of Kuopio and was in accordance with the Helsinki Declaration.

Laboratory methods

Plasma glucose and insulin were determined as previously described in detail (7). All four exons and the intron-exon junctions of the FABP2 gene were amplified with polymerase chain reaction using genomic DNA and the primers as previously reported (5). Single-strand conformation polymorphism (SSCP) analysis was performed essentially according to Orita et al. (8), described elsewhere in detail (9). Our method has been shown to detect all known mutants of the lipoprotein lipase gene (10). Variants were directly sequenced using Sequenase version 2.0 DNA polymerase (US Biochemicals, Cleveland, OH) (11).

Statistical analysis

Categorical variables between the study groups were compared with the χ^2 test and continuous variables with Student's two-tailed t test for independent samples. All data are presented as means \pm SE.

RESULTS— Table 1 summarizes the results of the screening with SSCP. In nondiabetic subjects with CHD, the SSCP analysis was performed in a random sample of 56 subjects. Additionally, the amino acid polymorphism in codon 54 of the FABP2 gene was studied in all 135 nondi-

From the Department of Medicine, University of Kuopio, Kuopio, Finland.

Address correspondence and reprint requests to Markku Laakso, MD, Department of Medicine, University of Kuopio, 70210 Kuopio, Finland. E-mail: markku.laakso@uku.fi.

Received for publication 13 October 1997 and accepted in revised form 26 January 1998.

Abbreviations: CHD, coronary heart disease; FABP, fatty acid-binding protein; SSCP, single-strand conformation polymorphism.

Table 1—Frequency of variants of the FABP2 gene in nondiabetic subjects and patients with NIDDM

	Nondiabetic subjects		Patients with NIDDM (CHD ⁺)
	CHD ⁻	CHD ⁺	
n	81	56	79
Exon 2			
GCT 54 → ACT 54	46 (0.28)	69 (0.26)*	42 (0.27)
Ala Thr			
Exon 4			
GTA 118 → GTG 118	46 (0.28)	37 (0.33)	36 (0.23)
3' noncoding region	8 (0.05)	2 (0.02)	5 (0.03)
GCGCA → GCACA			
Intron 2			
Number of ATT repeats			
9	0 (0.00)	0 (0.00)	1 (0.01)
10	85 (0.52)	51 (0.46)	87 (0.55)
11	26 (0.16)	24 (0.21)	30 (0.19)
12	2 (0.01)	0 (0.00)	1 (0.01)
13	34 (0.21)	27 (0.24)	26 (0.16)
14	14 (0.09)	10 (0.09)	13 (0.08)
15	1 (0.01)	0 (0.00)	0 (0.00)

Rare allele frequencies are given in parentheses. *Amino acid polymorphism in codon 54 was screened in all 135 nondiabetic subjects with CHD. CHD, coronary heart disease; CHD⁻, subjects without CHD; CHD⁺, subjects with CHD.

abetic subjects with CHD. The frequency of the threonine (Thr) encoding allele in codon 54 did not differ between the study groups. In addition, two other nucleotide substitutions were found (Table 1). In nondiabetic and NIDDM subjects, the allele frequencies of these variants as well as the allele frequencies of the ATT repeat sequence in intron 2 did not differ between the subjects with and without CHD or among all three groups.

Association of clinical characteristics and laboratory parameters were studied in nondiabetic and diabetic subjects (data not shown). No significant differences in BMI, systolic or diastolic blood pressure, or fasting plasma insulin or glucose concentrations were found between the subjects with the Thr54 allele compared with subjects with the Ala54Ala genotype in either the nondiabetic or diabetic groups.

CONCLUSIONS — The frequencies of the variants in the FABP2 gene, including the codon 54 polymorphism, did not differ between nondiabetic subjects with or without CHD or between NIDDM patients and control subjects. Furthermore, no association was found between the Thr54 allele and fasting insulin concentrations. These results imply that it is unlikely that the codon 54 polymorphism of the FABP2 gene affects insulin sensitivity.

In conclusion, we were unable to find gene defects in the FABP2 gene in nondiabetic and diabetic subjects with CHD. Therefore, the variants of the FABP2 gene are not significant determinants for the high risk of CHD in the Finnish population.

References

1. Schildkraut JM, Myers RH, Cupples LA,

Kiely DK, Kannel WB: Coronary risk associated with age and sex of parental heart disease in the Framingham Study. *Am J Cardiol* 64:555–559, 1989

2. Colditz GA, Rimm EB, Giovannucci E, Stampfer MJ, Rosner B, Willet WC: A prospective study of parental history of myocardial infarction and coronary artery disease in men. *Am J Cardiol* 67:933–938, 1991

3. Laakso M: Insulin resistance and coronary heart disease. *Curr Opin Lipidol* 7:217–226, 1996

4. Lowe JB, Sacchettini JC, Laposata M, McQuillan JJ, Gordon JL: Expression of rat intestinal fatty acid-binding protein in *Escherichia coli*: purification and comparison of ligand binding characteristics with that of *Escherichia coli*-derived rat liver fatty acid-binding protein. *J Biol Chem* 262:5931–5937, 1987

5. Baier LJ, Sacchettini JC, Knowler WC, Eads J, Paolisso G, Tataranni PA, Mochizuki H, Bennett PH, Bogardus C, Prochazka M: An amino acid substitution in the human intestinal fatty acid binding protein is associated with increased fatty acid binding, increased fat oxidation, and insulin resistance. *J Clin Invest* 95:1281–1287, 1995

6. World Health Organization: *Diabetes Mellitus: Report of a WHO Study Group*. Geneva, World Health Org., 1985 (Tech. Rep. Ser. no. 727)

7. Haffner SM, Karhapää P, Mykkänen L, Laakso M: Insulin resistance, body fat distribution, and sex hormones in men. *Diabetes* 43:212–219, 1994

8. Orita M, Iwahana H, Kanazawa H, Hayashi K, Sekiya T: Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms. *Proc Natl Acad Sci USA* 86:2766–2770, 1989

9. Laakso M, Malkki M, Kekäläinen P, Kuusisto J, Deeb SS: Insulin receptor substrate-1 variants in non-insulin-dependent diabetes. *J Clin Invest* 94:1141–1146, 1994

10. Nevin DN, Brunzell JD, Deeb SS: The LPL gene in individuals with familial combined hyperlipidemia and decreased LPL activity. *Arterioscler Thromb* 14:869–873, 1994

11. Kretz KA, Carson GS, O'Brien JS: Direct sequencing from low-melt agarose with Sequenase. *Nucleic Acids Res* 17:5864, 1989