Influence of Ala54Thr polymorphism of fatty acid-binding protein 2 on insulin resistance and adipocytokines in patients with diabetes mellitus type 2

D.A. DE LUIS, M. GONZALEZ SAGRADO, R. ALLER, O. IZAOLA, R. CONDE, B. DE LA FUENTE

Institute of Endocrinology and Nutrition, Medicine School and Unit of Investigation, Hospital Rio Hortega, University of Valladolid, Valladolid (Spain)

Abstract. – Background: A transition G to A at codon 54 of FABP2 results in an amino acid substitution (ala 54 to Thr 54). This polymorphism could be associated with insulin resistance and adipocytokines in patients with diabetes mellitus type 2.

Objective: The aim of our study was to investigate the influence of Thr54 polymorphism in the FABP2 gene on adipocytokines and insulin resistance in the fasted state in naïve patients with diabetes mellitus type 2.

Design: A population of 58 naïve patients with diabetes mellitus type 2 (fasting glucose >126 mg/dl) and obesity (body mass index >30) was analyzed in a prospective way. An indirect calorimetry, tetrapolar electrical bioimpedance, blood pressure, a serial assessment of nutritional intake with 3 days written food records and biochemical analysis (lipid profile, adipocytokines, insulin, CRP and HOMA) were performed. The statistical analysis was performed for the combined Ala54/Thr54 and Thr54/Thr54 as a mutant group and wild type Ala54/Ala54 as second group.

Results: Thirty-three patients (56.9%) had genotype Ala54/Ala54 (wild type group) and 25 (43.1%) patients Ala54/Thr54 (19 patients, 32.8%) or Thr54/Thr54 (6 patients, 10.3%) (mutant group). C-reactive protein (5.6±4.2 vs 8.7±5.3 mg/dl; p<0.05), insulin (22.9±11 vs 6.6±12 mUI/L; p<0.05) and HOMA (7.65±5.3 vs 9.1±6.3; p<0.05) were higher in mutant group than wild group. Anthropometric parameters, dietary intakes and adipocytokines were similar in both genotypes.

Conclusion: The novel finding of this study is the association of the Thr54/Ala54 and Thr54/Thr54 FABP2 genotypes with higher levels of C reactive protein, insulin and HOMA.

Key Words: Adipokines, Diabetes Mellitus, FABP2, Polymorphism, Insulin resistance.

Introduction

The current view of adipose tissue is that of an active secretory organ, sending out and responding to signals that modulate appetite, insulin sensitivity, energy expenditure, inflammation and immunity1-10.

The fatty acid (FA) binding protein 2 (FABP2) gene codes for intestinal FABP, which is a member of a family of small intracellular lipid-binding proteins. FABP plays an important role in several steps of unsaturated and saturated long chain fatty acids (LCFAs), protection of the cell from the cytotoxic effects of FFAs, and modulation of the enzyme additive involved in lipid metabolism11-12. Recently, a group13 reported a new G/A mutation. A transition G to A at codon 54 of FABP2 results in an amino acid substitution (Ala 54 to Thr 54). This polymorphism is common, with a Thr54 allelic frequency of 30% in most populations. This amino acid substitution was associated with high insulin resistance, and fasting insulin concentrations13. Carriers of the Thr54 allele have a 2-fold greater affinity for the long chain fatty acids than those with the Ala 54, which supports the role of the FABP2 Ala54Thr polymorphism in the etiology of metabolic disorders such as insulin resistance14.
The aim of our study was to investigate the influence of Thr54 polymorphism in the FABP2 gene on adipocytokines and insulin resistance in the fasted state in naïve patients with diabetes mellitus type 2.

Subjects and Methods

Subjects
A population of 58 naïve patients with diabetes mellitus type 2 (fasting glucose >126 mg/dl) and obesity (body mass index >30) was analyzed in a prospective way (research protocol accepted by the Ethical Committee). These patients were recruited in a Nutrition Clinic Unit and signed an informed consent. Exclusion criteria included history of cardiovascular disease or stroke during the previous 36 months, total cholesterol >300 mg/dl, triglycerides >400 mg/dl, blood pressure >140/90 mmHg, as well as the use of sulphonylureas, thiazolidinediones, metformin, insulin, glucocorticoids, antineoplastic agents, angiotensin receptor blockers, angiotensin converting enzyme inhibitors, psychoactive medications, drinking and/or smoking habit.

Procedure
All patients with a 2 weeks weight-stabilization period before recruitment were enrolled. Weight, blood pressure, basal glucose, c-reactive protein (CRP), insulin, insulin resistance (HOMA), total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides blood and adipocytokines (leptin, adiponectin, resistin, TNF-alpha, and interleukin 6) levels were measured at basal time. A tetrapolar bioimpedance, an indirect calorimetry and a prospective serial assessment of nutritional intake with 3 days written food records were realized. Genotype of FABP2 gene polymorphism was studied.

Genotyping of FAPP2 Gene Polymorphism
Oligonucleotide primers and probes were designed with the Beacon Designer 4.0 (Premier Biosoft International®, LA, CA). The polymerase chain reaction (PCR) was carried out with 50 ng of genomic DNA, 0.5 µL of each oligonucleotide primer (primer forward: 5’-CAG TTC CGT CTG CTA GAT TGT-3’; primer reverse: 5’-GCT GAC AAT TAC ACA AGA AGG AA-3’), and 0.25 µL of each probes (wild probe: 5’-Fam-CAA AGA ATC AAG CAC TTT TCG AAA CA-BHQ-1-3’) and (mutant probe: 5’-Hex-AGA ATC AAG CGC TTT TCG AAA CA-BHQ-1-3’) in a 25 µL final volume (Termociclador iCycler IQ (Bio-Rad®), Hercules, CA). DNA was denatured at 95°C for 3 min; this was followed by 50 cycles of denaturation at 95°C for 15 s, and annealing at 59.3°C for 45 s). The PCR were run in a 25 µL final volume containing 12.5 µL of IQTM Supermix (Bio-Rad®, Hercules, CA) with hot start Taq DNA polymerase. Hardy Weimberger equilibrium was assessed.

Assays
Serum total cholesterol and triglyceride concentrations were determined by enzymatic colorimetric assay (Technicon Instruments, Ltd., New York, N.Y.), while HDL cholesterol was determined enzymatically in the supernatant after precipitation of other lipoproteins with dextran sulfate-magnesium. LDL cholesterol was calculated using Friedewald formula.

Haemoglobin A1c levels were measured by using high-pressure liquid chromatography. Plasma glucose levels were determined by using an automated glucose oxidase method (Glucose analyser 2, Beckman Instruments, Fullerton, CA). Insulin was measured by RIA (RIA Diagnostic Corporation, Los Angeles, CA) with a sensitivity of 0.5 mUI/L (normal range 0.5-30 mUI/L)15 and the homeostasis model assessment for insulin sensitivity (HOMA) was calculated using these values16. CRP was measured by immunoturbimetry (Roche Diagnostcis GmbH, Mannheim, Germany), with a normal range of (0-7 mg/dl) and analytical sensitivity 0.5 mg/dl.

Adipocytokines
Resistin was measured by ELISA (Biovendor Laboratory, Inc., Brno, Czech Republic) with a sensitivity of 0.2 ng/ml with a normal range of 4-12 ng/ml17. Leptin was measured by ELISA (Diagnostic Systems Laboratories, Inc., Minneapolis, MN) with a sensitivity of 0.05 ng/ml and a normal range of 10-100 ng/ml18. Adiponectin was measured by ELISA (R&D Systems, Inc., Minneapolis, MN) with a sensitivity of 0.246 ng/ml and a normal range of 8.65-21.43 ng/ml19. Interleukin 6 and TNF alpha were measured by ELISA (R&D Systems, Inc., Minneapolis, MN) with a sensitivity of 0.7 pg/ml and 0.5 pg/ml, respectively. Normal values of IL6 was (1.12-12.5 pg/ml) and TNFalpha (0.5-15.6 pg/ml)20-21.
Indirect Calorimetry
For the measurement of resting energy expenditure, subjects were admitted to a metabolic ward. After a 12 h overnight fast, resting metabolic rate was measured in the sitting awake subject in a temperature-controlled room over one 20 min period with an open-circuit indirect calorimetry system (standardized for temperature, pressure and moisture) fitted with a face mask (MedGem; Health Tech, Golden, USA), coefficient of variation 5%. Resting metabolic rate (kcal/day) and oxygen consumption (ml/min) were calculated.

Anthropometric Measurements
Body weight was measured to an accuracy of 0.5 kg and BMI computed as body weight/(height^2). Waist (narrowest diameter between xiphoid process and iliac crest) and hip (widest diameter over greater trochanters) circumferences to derive waist-to-hip ratio (WHR) were measured, too. Tetrapolar body electrical bioimpedance was used to determine body composition with an accuracy of 5 g. An electric current of 0.8 mA and 50 kHz was produced by a calibrated signal generator (Biodynamics Model 310e, Seattle, WA) and applied to the skin using adhesive electrodes placed on right-side limbs. Resistance and reactance were used to calculate total body water, fat and fat-free mass.

Blood pressure was measured twice after a 10 minutes rest with a random zero mercury sphygmomanometer, and averaged.

Dietary Intake and Habits
Patients received prospective serial assessment of nutritional intake with 3 days written food records. All enrolled subjects received instruction to record their daily dietary intake for three days including a weekend day. Handling of the dietary data was by means of a personal computer equipped with personal software, incorporating use of food scales and models to enhance portion size accuracy. Records were reviewed by a registered dietitian and analyzed with a computer-based data evaluation system. National composition food tables were used as reference.

Statistical Analysis
Sample size was calculated to detect differences over 5% in insulin resistance with 90% power and 5% significance. The results were expressed as mean ± SD. The distribution of variables was analyzed with Kolmogorov-Smirnov test. Quantitative variables with normal distribution were analyzed with a two-tailed Student’s-t test. Non-parametric variables were analyzed with the U-Mann-Whitney test. Qualitative variables were analyzed with the chi-square test, with Yates correction as necessary, and Fisher’s test. The statistical analysis was performed for the combined Ala54/Thr54 and Thr54/Thr54 as a group and wild type Ala54/Ala54 as second group. Anova test was used to analyzed quantitative variables with three groups (Ala54/Thr54, Thr54/Thr54 and Ala54/Ala54 patient groups). A p-value under 0.05 was considered statistically significant.

Results
Fifty eight patients gave informed consent and were enrolled in the study. The mean age was 57.4±11.7 years and the mean BMI 37.8±6.5, with 15 males and 43 females.

Thirty-three patients (56.9%) had genotype Ala54/Ala54 (wild type group) and 25 (43.1%) patients Ala54/Thr54 (19 patients, 32.8%) or Thr54/Thr54 (6 patients, 10.3%) (mutant group). Age was similar in both groups (wild type: 57.3±11.7 yrs vs mutant group: 56.1±11 yrs: ns). Sex distribution was similar in both groups (wild vs mutant group), males (27.3% vs 24%) and females (72.7% vs 76%).

Table I shows anthropometric variables, without statistical differences. Table II shows cardiovascular risk factors. C-reactive protein, insulin and HOMA were higher in mutant group than wild group.

Table III shows nutritional intake with 3 days written food records. Calory, carbohydrate, fat, and protein intakes were similar in both groups, without differences between allelic groups.

Table IV shows levels of adipocytokines. No differences were detected between genotypes.

Discussion
The novel finding of this study is the association of the Thr54/Ala54 and Thr54/Thr54 FABP2 genotypes with higher levels of insulin, HOMA and C reactive protein.

One hypothesis to explain these data has been shown in a previous study. Baier et al. concluded
that threonine-containing protein may increase absorption and/or processing dietary fatty acids by the intestine and therefore increase fat oxidation, which has been shown to inhibit glucose uptake in muscle and results in insulin resistance.

However, contradictory results have been described in the literature. Sipilainen et al.25 found that obesity is not associated with specific variants in the FABP2 gene and that the Ala54-to-Thr polymorphism did not influence insulin levels in obese Finns.

The frequency of the Thr54 allele variant is 43% in our study. Slightly lower frequency have been reported for other population as non diabetic Pima Indians (30%), Koreans (34%), Japanese (35%), Swedish (30%) and white individuals from USA (32%)26-30. Perhaps, these different frequencies and outcomes could be explained by inclusion criteria of subjects in previously studies of the literature. For example, Carlsson et al31 have detected higher concentrations of triglyceride and cholesterol in the Thr54 allele patients, in a population of obese patients with parenteral history of cardiovascular disease. In a population of type 2 diabetes mellitus32, a linear relationship of mean fasting plasma triglyceride levels was found and after fat ingestion, in homozygous for the Thr54 allele than in wildtype patients. In type 1 diabetes mellitus patients33 do not interact with the codon 54 polymorphism of the FABP2 gene to cause dyslipemia. Other population association studies with insulin resistance and lipids in patients with type 2 diabetes mellitus34,35 were essentially negative. Perhaps, the type of diabetes, the time course of this disease, pharmacological treatments received by patients and comorbidities

Table I. Anthropometric variables.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Ala54/Ala54 (n = 33)</th>
<th>(Ala54/Thr54 or Thr54/Thr54) (n = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>37.4 ± 7.6</td>
<td>37.4 ± 5.3</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>94.7 ± 20.4</td>
<td>94.8 ± 15</td>
</tr>
<tr>
<td>Fat free mass (kg)</td>
<td>53.1 ± 14.9</td>
<td>51.2 ± 14.3</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>40.6 ± 17</td>
<td>43.6 ± 14.8</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>112.1 ± 14</td>
<td>118.9 ± 13.7</td>
</tr>
<tr>
<td>Waist to hip ratio</td>
<td>0.96 ± 0.1</td>
<td>0.97 ± 0.09</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>135.4 ± 21.1</td>
<td>138.5 ± 19</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>83.1 ± 9.1</td>
<td>81.4 ± 9.1</td>
</tr>
<tr>
<td>RMR (kcal/day)</td>
<td>1938 ± 421</td>
<td>2100 ± 805</td>
</tr>
</tbody>
</table>

RMR: resting metabolic rate. WC: Waist circumference. (*) p<0.05, in each group with basal values.

Table II. Classical cardiovascular risk factors.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Ala54/Ala54 (n = 33)</th>
<th>(Ala54/Thr54 or Thr54/Thr54) (n = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>132.8 ± 28</td>
<td>131.8 ± 22</td>
</tr>
<tr>
<td>Total ch. (mg/dl)</td>
<td>211 ± 38</td>
<td>220 ± 44</td>
</tr>
<tr>
<td>LDL-ch. (mg/dl)</td>
<td>138.4 ± 39</td>
<td>144.9 ± 36</td>
</tr>
<tr>
<td>HDL-ch. (mg/dl)</td>
<td>51.4 ± 10.5</td>
<td>52.7 ± 11.6</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>152.6 ± 58</td>
<td>163 ± 59</td>
</tr>
<tr>
<td>Insulin (mU/L)</td>
<td>22.9 ± 11</td>
<td>26.6 ± 12*</td>
</tr>
<tr>
<td>HOMA</td>
<td>7.65 ± 5.3</td>
<td>9.1 ± 6.3*</td>
</tr>
<tr>
<td>CRP (mg/dl)</td>
<td>5.6 ± 4.2</td>
<td>8.7 ± 5.3*</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.8 ± 0.9</td>
<td>5.5 ± 0.7</td>
</tr>
</tbody>
</table>

Ch: Cholesterol, TG: Triglycerides CRP: c reactive protein. HOMA: Homeostasis model assessment. (*) p<0.05, in each group with basal values.
could influence in these unclear previous results. We propose studies with naïve diabetic patients as our design.

The lack of association with cholesterol and triglyceride levels is clear in our study. Other authors have shown higher levels of cholesterol and triglycerides in non-diabetic obese patients with Thr allele. Nevertheless, Duarte et al have shown a lower total and LDL cholesterol levels in patients with Thr allele. These previous studies would have required composition analysis of the dietary intake to determine whether dietary components could be responsible for the lipid profile modifications. For example, Marin et al have shown that insulin sensitivity decreased in subjects with Thr54 allele of the FABP2 polymorphism when saturated fatty acids were replaced by monounsaturated fatty acids and carbohydrates. In our study dietary intake did not show statistical differences between groups, in this way our data have been controlled by dietary intake and previous discrepancies could be explained by this uncontrolled factor (dietary intake).

In our study, the elevation of inflammation markers (CRP) in patients with Thr allele, as shown in previous studies could be related with an altered postprandial response of fatty acids absorption (no measured in our design). Elevated FFA increases the accumulation of triglycerides in the adipocyte, related with imbalance of lipoprotein lipase activity. The association of this proinflammatory state with insulin resistance in Th carriers could indicate the existence of complex gene-gene or gene-environment interactions that may enhance metabolic abnormalities in these patients.

In conclusion, the novel finding of this study is the association of the Thr54/Ala54 and Thr54/Thr54 FABP2 genotypes with higher levels of C reactive protein, insulin and HOMA. Further studies are needed to elucidate the complex relationships among lipoprotein metabolism, fat storage, inflammation markers and Thr allele. A role of Ala54Thr polymorphism on incidence of diabetes mellitus type 2 might be explored as a new association.

### Table III. Dietary intake.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Ala54/Ala54 (n = 33)</th>
<th>Ala54/Thr54 or Thr54/Thr54 (n = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal/day)</td>
<td>1682 ± 555</td>
<td>1550 ± 497</td>
</tr>
<tr>
<td>CH (g/day)</td>
<td>171.5 ± 72</td>
<td>160.7 ± 66</td>
</tr>
<tr>
<td>Fat (g/day)</td>
<td>72.9 ± 30</td>
<td>66.7 ± 36</td>
</tr>
<tr>
<td>S-fat (g/day)</td>
<td>19.9 ± 11</td>
<td>17.1 ± 9.2</td>
</tr>
<tr>
<td>M-fat (g/day)</td>
<td>35.1 ± 14.3</td>
<td>30.9 ± 16.5</td>
</tr>
<tr>
<td>P-fat (g/day)</td>
<td>6.6 ± 3.2</td>
<td>5.6 ± 3.4</td>
</tr>
<tr>
<td>Protein (g/day)</td>
<td>82.4 ± 21</td>
<td>78.9 ± 21</td>
</tr>
<tr>
<td>Exercise (hs./week)</td>
<td>1.45 ± 2.8</td>
<td>1.38 ± 2.9</td>
</tr>
<tr>
<td>Dietary fiber</td>
<td>15.3 ± 6.1</td>
<td>14.7 ± 6.5</td>
</tr>
</tbody>
</table>


### Table IV. Circulating adipocytokines.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Ala54/Ala54 (n = 33)</th>
<th>Ala54/Thr54 or Thr54/Thr54 (n = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL 6 (pg/ml)</td>
<td>3.34 ± 2.2</td>
<td>3.1 ± 2.5</td>
</tr>
<tr>
<td>TNF-alpha (pg/ml)</td>
<td>6.79 ± 3.8</td>
<td>6.4 ± 4.2</td>
</tr>
<tr>
<td>Adiponectin (ng/ml)</td>
<td>33.3 ± 30</td>
<td>28.5 ± 23</td>
</tr>
<tr>
<td>Resistin (ng/ml)</td>
<td>3.4 ± 1.8</td>
<td>3.7 ± 1.9</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>82.7 ± 66</td>
<td>108.4 ± 68</td>
</tr>
</tbody>
</table>

IL-6: interleukin 6. (*) p<0.05, in each group with basal values.
References


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