Int J Clin Exp Med 2015;8(9):16389-16393
www.ijcem.com /ISSN:1940-5901/IJCEM0013265

Original Article

Association of I-FABP gene polymorphism and the risk of coronary heart disease

Dong Yuan[1,2], Changqing Yu[1,2], Chunyu Zeng[1,2]

[1]*Department of Cardiology, Daping Hospital, The Third Military Medical University, Chongqing 400042, China;*
[2]*Chongqing Institute of Cardiology, Chongqing 400042, China*

Received July 22, 2015; Accepted September 3, 2015; Epub September 15, 2015; Published September 30, 2015

Abstract: Objective: The study aimed to investigate the association between polymorphism of I-FABP gene and coronary heart disease (CHD). Methods: 225 patients with CHD were randomly recruited into the case group, and 196 healthy elderly volunteers were recruited from Medical Examination Center of our hospital as control. General clinical data were collected and plasma TC, TG, LDL-C, HDL-C levels were measured. Besides, polymerase chain reaction (PCR) and Restriction Fragment Length Polymorphism (RFLP) technology were used to detect the polymorphism of Hha-I enzyme cleavage sites in I-FABP gene in the study population. Results: Hha-I cleavage sites occurred at codon 54 in exon in the coding sequencing of I-FABP gene in all participants. After cleavage with Hha-I enzyme, the genotypes were identified as wild-type A/A, heterozygous mutant A/T and homozygous mutant T/T. In case group, A/T and T/T genetic carriers had significantly higher levels of TC, TG and LDL-C than A/A carriers ($P<0.05$). However, in control group, similar differences were not observed ($P>0.05$). BMI, dietary habits and I-FABP alleles were independent risk factors of CHD. Conclusion: The polymorphism of I-FABP gene existed in the study population. And this genetic variation had influence on lipid metabolism, which was associated with the risk of developing CHD. I-FABP gene polymorphism may contribute to the increased genetic susceptibility to CHD.

Keywords: Fatty acid binding proteins, coronary heart disease, dyslipidemia, PCR, alleles, risk factors

Introduction

Reducing the incidence and mortality is crucial in prevention and treatment of coronary heart disease (CHD). And addressing its risk factors has positive significance. Apart from well-established risk factors such as smoking, dyslipidemia and hypertension, studies have investigated potential role of genetic mutation in developing CHD [1-4].

I-FABP, a 15 kDa molecule secreted by intestinal single-layered column epithelial cells, binds to long-chain fatty acid (LCFA) and plays an important role in its uptake, transportation and metabolic regulation [5]. The main function of I-FABP is to transport hydrophobic fatty acids from cell membrane to endoplasmic reticulum, where the fatty acids react with glycerol 3-phosphate to form TGs. When TGs are assembled with apoproteins, chylomicrons are formed and then transported into blood circulation via lymphatic duct. From the perspective of structure and equivalence principle, the transportation ability of I-FABP is related to its binding affinity for LCFA, which decreases with increased length of LCFA. Besides, I-FABP has higher affinity for saturated fatty acids than non-saturated ones [6-8].

The function of I-FABP is closely related to its structure, whose tertiary structure is like one open-ended barrel. I-FABP comprises two α-helices and one β-sheet. The conformation of barrel-like structure consists of 10 antiparallel β-strands, which form two beta-sheets of five strands each and then organize into a local flat clam called “β-clam”. Two α-helices are located at one side, closing one end of β-clam, while the other end is open, forming so-called “opening” structure. The interior of β-clam resembles a shell clam, and this is where the ligand binds. Fifty percent of residual chains in the interior are hydrophobic. However, the core is formed by a five-membered hydrogen bonding network, consisting of Arg-127, Glnll5 and water mole-

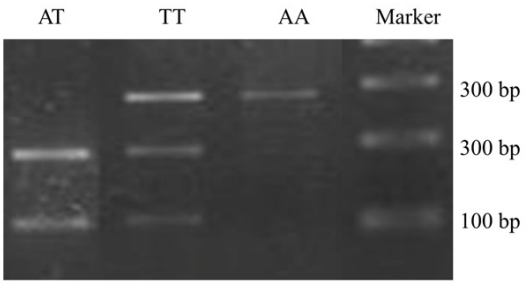

Figure 1. Genotypes of I-FABP gene by PCR-RFLP.

cules. Binding state does not influence the molecular conformation. When fatty acid binds, only one fatty acid and seven water molecules are accommodated in the ligand-binding domain; when fatty acid is released, 13 water molecules occupy the domain. The guanidinium group of Arg-56, the only amino-group oriented towards the exterior, and the guanidinium group of Arg-20, which points to the interior, form hydrogen bonds with Tyr-70, Glu-51 and Ser 71 to maintain the 3-D dimensional structure of protein [9-11].

I-FABP is one of these candidate genes, and its polymorphism at codon 54 has been studied extensively. A growing body of evidence shows that mutation of I-FABP has influence on lipid metabolism, especially TG and LDL-C, which leads to dyslipidemia. Our study aimed to investigate the association between polymorphism of I-FABP gene and lipid metabolism as well as the risk of CHD by amplifying the exon of I-FABP gene in CHD patients.

Material and methods

Participant selection

225 patients with CHD visiting our hospital from June 2008 to Nov 2012 were recruited and divided into case group. Among them, 124 patients were male, and 101 were female. The average age was 60.5±7.6 years. 196 healthy volunteers from Medical Examination Center in Daping Hospital, The Third Military Medical University, 108 males and 88 females, were recruited and classified into control group. The average age was 60.8±6.4 years. Informed consent was obtained from all participants. And patient information including height, BMI, gender, smoking and drinking history, dietary habits, accompanying hypertension and diabetes was recorded.

Inclusion criteria: CHD was diagnosed after careful history taking, thorough physical examination and positive coronary arteriography indicating greater than 50% coronary artery stenosis, according to diagnostic criteria of CHD published by WHO in 1979.

Exclusion criteria: Participants with diabetes, recent myocardial infarction, heart failure, blood pressure higher than 160/100 mmHg and accompanying severe organic disorders involving brain, liver and kidney, and lower extremity dysfunction were excluded.

Methods

Chemicals and materials: Genomic DNA extraction kit was obtained from Tiangen Biotech (Beijing) Co., Ltd for blood, tissue and cell analysis. Super Taq PCR Mix was purchased from Boerdi Biotech Company (Nanjing, China). 1% agarose and Hha I restriction enzyme were obtained from Jingmei Company (USA). DNA primers were designed with primer 5.0 software according to literature and published primers for human gene sequences.

Forward primer: 5'-ACAGGTGTTAATATAGTGAAAAGG-3', reverse primer: 5'-ATTGGCTTCTTCAGTTAGTGAAGG-3'. These primers were further produced by Sangon Biotech (Shanghai, China). DNA biomarkers and Bio-Rad gradient PCR machine were also obtained from Sangon Biotech.

Experimental methods: Three to four milliliters of venous blood was drawn from each participant in both groups after a twelve hour fast. Blood sample was distributed into two EDTA-containing tubes for PCR and plasma lipid panel.

Blood was centrifuged and serum was removed. Olympus fully automatic biochemistry analyzer was used to test the levels of TC, TG, LDL-C and HDL-C in plasma.

Genotyping: The target gene was extracted according to protocols of DNA extract kit. The extracted DNA sample was electrophoresed in 1% agarose gel and photographed under ultraviolet light to determine the purity.

For the PCR, DNA samples (2 mL) were amplified in a 30- mL volume consisting of 25 mL 2×

Table 1. The distribution of genotype and allele of I-FABP

Group	N	Genotype [n, (%)]			P	Allele [n, (%)]		P
		TT	AT	AA		T	A	
Control	196	65 (33.2)	81 (41.3)	50 (25.5)	<0.001	211 (53.8)	181 (46.2)	<0.001
Case	225	122 (54.2)	88 (39.1)	15 (6.7)		332 (73.7)	362 (26.3)	

Taq Master Mix, 10 pmol of each primer and 21 mL Nuclease-Free water. The PCR program consisted of an initial denaturation step at 94°C for 5 min followed by 25 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 40 s. The final extension step was performed at 72°C for 5 min. 5 mL PCR products were mixed with 1 mL loading buffer, electrophoresed in 1.5% agarose gel and photographed under ultraviolet light.

For Restriction Fragment length Polymorphism (RFLP) analysis, 1 mL PCR products were incubated with 1 mLl of enzyme Hha-I, 16 μL of Nuclease-Free water and 2 μl of 10× Tango buffer overnight at 37°C in incubator. 5 μl products were mixed with 1 μl loading buffer, run on 2% agarose gel containing 0.3 mL/mL nucleic acid dye, electrophoresed and observed under ultraviolet light. Genotype was determined by analyzing Hha-I digested PCR products.

Statistical analysis

SPSS software 16.0 was used for data analysis. Quantitative data were represented in the form of Mean ± SD. t-test was used for comparing quantitative data between groups, which were subject to normal or approximate normal distribution. Genotype frequencies were calculated by direct counting. Hardy-Weinberg Equilibrium was evaluated using a fitting Chi square test. A step-wise multiple logistic regression model was used to control cofounding factors and identify interaction between factors. *P* value less than 0.05 was considered statistically significant.

Results

SNP of I-FABP gene

As shown in **Figure 1**, I-FABP gene contained Hha-I cleavage sites. After digestion with Hha-I, DNA of wild type A/A only had one fragment (293 bp), DNA of homozygous mutant cleavages into two fragments (216 bp and 77 bp), while DNA of heterozygous mutant was split into three fragments (293 bp, 216 bp and 77 bp).

Hardy-Weinberg equilibrium test

The genotype distribution was in line with the Hardy-Weinberg equilibrium both in the case and control group (Both P>0.05).

Genotype distribution between case and control group

As shown in **Table 1**, the frequency of T alleles in the case group was higher than that in the control group (P<0.001), and TT genotype was common in the case group than that in the control group (P<0.001).

Association between plasma lipid level and polymorphism of I-FABP gene in case and control group

In case group, the carriers of T allele (AT and TT genotype) had significantly higher TC, TG, and LDL-C than non-carriers (AA genotype) (all P<0.01). No difference was found in HDL-C level between T allele carriers and non-carriers (P>0.05). However, there were no significant differences in the TC, TG, LDL-C and HDL-C levels between T allele carriers and non-carriers in control group (**Table 2**).

Logistic regression analysis for risk factors

Gender, BMI, drinking history, dietary habits and genotype of I-FABP were considered as candidate variables for the multivariate logistic regression model. And the results showed that BMI, dietary habits and I-FABP alleles were independent risk factors for coronary artery disease (**Table 3**).

Discussions

I-FABP gene is located in the long arm of chromosome 4 at 4q28-q31, with the length of about 3.4 Kb. It contains four exons, interrupted by four introns. The G to A polymorphism of codon 54 in exon 2 results in the substitution

Table 2. Lipids level in different genotype between the case and control group

Groups	N	TG (mmol/L)	TC (mmol/L)	LDL-C (mmol/L)	HDL-C (mmol/L)
Case group					
AA	15	1.89±0.71	5.01±0.77	2.64±0.56	1.11±0.57
AT	88	2.88±0.92*	6.82±0.91*	3.45±0.72*	1.25±0.71
TT	122	3.24±1.02*	6.94±0.96*	3.78±1.12*	1.30±1.01
Control group					
AA	50	1.91±0.66	5.44±0.35	2.77±0.76	1.03±0.51
AT	81	1.98±0.91	5.81±0.98	3.01±0.88	1.20±0.67
TT	65	2.14±0.95	6.01±1.03	3.14±0.98	1.29±0.89

*P<0.05, compared to AA genotype.

Table 3. Logistic regression of the relation between I-FABP polymorphism and CHD

Parameters	OR	95% CI	*P* value
I-FABP polymorphism	1.71	1.120~3.112	0.012
Smoking	2.344	1.124~5.212	0.025
BMI	1.233	1.014~3.165	0.024
Dietary habits	1.667	0.775~3.135	0.421
Drinking	0.673	0.115~1.091	0.078

of a threonine for an alanine and leads to changes in tertiary structure of I-FABP, especially conformation of ligand-binding domain. This influences the binding properties of I-FABP for LCFA. Subsequent studies show that polymorphism at this very site is associated with dysregulated lipid metabolism, insulin resistance, increased risk of CHD, type II diabetes and its related complications [12].

Our study showed that T allele carriers had a significantly higher level of TC, TG and LDL-C than non-carriers in case group. However, a similar trend was not found in control group. This finding indicated that abnormalities of lipid metabolism existed in CHD patients with AT and TT genotype, which was consistent with previous reports. Increased absorption of LCFA by mutant I-FABP and subsequent increased level of TG may account for the lipid metabolism abnormalities. In control group, a lack of difference in the lipid level despite the mutant I-FABP may be associated with lower frequency of mutant alleles, less intake of LCFA, a healthier dietary habit and appropriate sodium and fat intake. This indicated a possible interaction between dietary habits and genetic mutation, which needed to be further explored in the future.

CHD is a prevalent chronic disease in elderly population and has characteristics such as atypical symptoms and high mortality. Research has been focusing on its secondary prevention, development risk and possible risk factors, especially gene mutations. The genetic variation may contribute to variation in disease susceptibility and play a vital role in risk prediction, primary prevention and early diagnosis.

Our study also introduced gender, BMI, drinking history, dietary habits and I-FABP alleles as candidate variables into a multiple logistic regression model, and identified independent risk factors using step-wise regression selection. The results showed I-FABP alleles, BMI and smoking were independent risk factors for CHD, consistent with previous studies. Carriers with T allele in the I-FABP gene had increased intake of TG and disrupted fatty acid oxidation, which resulted in dyslipidemia and increased risk of CHD attacks.

In conclusion, our study revealed that I-FABP gene polymorphism had effect on lipid metabolism of elderly CHD patients. And this genetic variation could be used as a screening risk factor to predict risk of CHD attack in elderly patients, which had positive significance in preventing and treating CHD at a molecular level.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Chunyu Zeng, Department of Cardiology, Daping Hospital, The Third Military Medical University; Chongqing Institute of Cardiology, No. 10 Changjiangzhilu, Chongqing 400042, China. Tel: +8602368757001; E-mail: lyjbs87@126.com

References

[1] Park JH, Bae SH. A systematic review of psychological distress as a risk factor for recurrent cardiac events in patients with coronary artery disease. J Korean Acad Nurs 2011; 41: 704-14.

[2] Uddin SN, Begum F, Malik F, Rahman S. Coronary artery disease in young patients: clin-

ical review and risk factor analysis. Mymensingh Med J 2003; 12: 3-7.
[3] Dytfeld D, Leśna J, Protasewicz A, Sarnowski W, Dyszkiewicz W, Paradowski S. Ear lobe crease as a factor of potential risk for coronary artery disease? -World news review and own research. Pol Arch Med Wewn 2002; 108: 633-8.
[4] Herrera CR, Lynch C. Is baldness a risk factor for coronary artery disease? A review of the literature. J Clin Epidemiol 1990; 43: 1255-60.
[5] Gonçalves FL, Soares LM, Figueira RL, Simões AL, Gallindo RM, Sbragia L. Evaluation of the expression of I-FABP and L-FABP in a necrotizing enterocolitis model after the use of Lactobacillus acidophilus. J Pediatr Surg 2015; 50: 543-9.
[6] Sarikaya M, Ergül B, Doğan Z, Filik L, Can M, Arslan L. Intestinal fatty acid binding protein (I-FABP) as a promising test for Crohn's disease: a preliminary study. Clin Lab 2015; 61: 87-91.
[7] Khadaroo RG, Fortis S, Salim SY, Streutker C, Churchill TA, Zhang H. I-FABP as biomarker for the early diagnosis of acute mesenteric ischemia and resultant lung injury. PLoS One 2014; 9: e115242.
[8] Voth M, Holzberger S, Auner B, Henrich D, Marzi I, Relja B. I-FABP and L-FABP are early markers for abdominal injury with limited prognostic value for secondary organ failures in the post-traumatic course. Clin Chem Lab Med 2015; 53: 771-80.
[9] Her GM, Chiang CC, Wu JL. Zebrafish intestinal fatty acid binding protein (I-FABP) gene promoter drives gut-specific expression in stable transgenic fish. Genesis 2004; 38: 26-31.
[10] Geschonke K, Klempt M, Lynch N, Schreiber S, Fenselau S, Schrezenmeir J. Detection of a promoter polymorphism in the gene of intestinal fatty acid binding protein (I-FABP). Ann N Y Acad Sci 2002; 967: 548-53.
[11] Le Beyec J, Delers F, Jourdant F, Schreider C, Chambaz J, Cardot P, Pinçon-Raymond M. A complete epithelial organization of Caco-2 cells induces I-FABP and potentializesapolipoprotein gene expression. Exp Cell Res 1997; 236: 311-20.
[12] Geschonke K, Klempt M, Lynch N, Schreiber S, Fenselau S, Schrezenmeir J. Detection of a promoter polymorphism in the gene of intestinal fatty acid binding protein (I-FABP). Ann N Y Acad Sci 2002; 967: 548-53.