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Association between Thr312Ala Polymorphism in α-Fibrinogen Gene and Coronary Artery Disease*

Związek między polimorfizmem Thr312Ala w genie α-fibrynogenu a chorobą niedokrwienną serca

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Abstract

Background. Fibrinogen is an acute phase protein synthesized in the liver and its increased plasma level may reflect the inflammatory condition of the vascular wall and thus may be related to cardiovascular risk. Human fibrinogen is composed of three pairs of polypeptide chains: α, β, γ coded by three different genes. A common polymorphism in α-chain fibrinogen (*FGA*) gene leads to an amino acid substitution from threonine to alanine at codon 312 and occurs within the carboxy-terminal end of α-chain.
Objectives. The aim of the study was to evaluate the association of the Thr312Ala polymorphism in *FGA* gene with coronary artery disease (CAD) in Polish population.
Material and Methods. We studied group of 147 white Caucasians including 74 angiographically documented CAD patients aged 46.5 ± 6.2 years and 73 healthy blood donors aged 35.2 ± 9.8 years. The Thr312Ala polymorphism was detected using polymerase chain reaction and restriction digestion with *Rsa*I enzyme.
Results. We found higher frequency of A allele of Thr312Ala polymorphism in patients group with coronary artery disease than in controls (p = 0.049, OR = 1.78). Carriers of A allele (TA+AA genotypes) appeared also significantly more often in cases compared to control group (p = 0.031, OR = 2.10). Number of carriers of A allele was especially high in subgroup of male, hypertensive and overweight patients.
Conclusions. Present study suggests association between Thr312Ala polymorphism in *FGA* gene and coronary artery disease in Polish population (**Adv Clin Exp Med 2005, 14, 3, 445–449**).

Key words: gene polymorphism, α-fibrinogen, coronary artery disease.

Streszczenie

Wprowadzenie. Fibrynogen jest białkiem ostrej fazy syntetyzowanym w wątrobie. Podwyższone stężenie fibrynogenu w osoczu może wskazywać na stan zapalny śródbłonka i dlatego jest potencjalnym czynnikiem ryzyka choroby niedokrwiennej serca. Ludzki fibrynogen jest zbudowany z trzech par łańcuchów polipeptydowych: α, β, γ, kodowanych przez trzy różne geny. Powszechny polimorfizm w genie łańcucha α fibrynogenu (*FGA)* prowadzi do zamiany treoniny na alaninę w kodonie 312 w obrębie końca C łańcucha α.
Cel pracy. Ocena związku polimorfizmu Thr312Ala w genie *FGA* z chorobą niedokrwienną serca w populacji polskiej.
Materiał i metody. Badania przeprowadzono na grupie 147 osób rasy kaukaskiej, w tym 74 pacjentów z koronarograficznie potwierdzoną chorobą niedokrwienną serca w wieku 46,5 ± 6,2 lat i 73 zdrowych krwiodawców w wieku 35,2 ± 9,8 lat, bez obciążeń chorobami sercowo-naczyniowymi w wywiadzie. Polimorfizm Thr312Ala analizowano metodą reakcji łańcuchowej polimerazy, a następnie trawiono enzymem restrykcyjnym *Rsa*I.
Wyniki. Stwierdzono wyższą częstość występowania allelu A polimorfizmu Thr312Ala w grupie pacjentów z potwierdzoną chorobą niedokrwienną serca niż w grupie kontrolnej (p = 0,049, OR = 1,78). Nosiciele allelu A (genotypy TA+AA) występowali również znamiennie częściej wśród chorych w porównaniu z grupą zdrowych (p = 0,031, OR = 2,10). Liczba nosicieli allelu A wśród pacjentów była szczególnie duża w podgrupie pacjentów płci męskiej, ze stwierdzonym nadciśnieniem tętniczym i nadwagą.
Wnioski. Wyniki badań wykazały związek między polimorfizmem Thr312Ala w genie *FGA* a chorobą niedokrwienną serca w populacji polskiej (**Adv Clin Exp Med 2005, 14, 3, 445–449**).

Słowa klucze: polimorfizm genetyczny, α-fibrynogen, choroba niedokrwienna serca.

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Fibrinogen is an acute phase protein synthesized in the liver and its increased plasma level may reflect the inflammatory condition of the vascular wall and thus may be related to cardiovascular risk. Fibrinogen is the last target in coagulation cascade and its lysis by thrombin gives soluble fibrin fragments stabilized by factor XIII in a clot. Human fibrinogen is a dimeric glycoprotein composed of three pairs of polypeptide chains: α, β, γ which are linked by disulfide bonds. These chains are coded by three different genes clustered on a long arm of chromosome 4q2 [1]. Several polymorphisms in genes encoding fibrinogen chains have been described but the most studied are located in β-chain gene because it is suggested that the rate of production of mature fibrinogen is limited by synthesis of β-chain [2].

A common polymorphism in α-chain fibrinogen (*FGA*) gene leads to an amino acid substitution from threonine to alanine at codon 312 and occurs within the carboxy-terminal end of α-chain [3]. The Thr312Ala polymorphism lies near to the fibrin α/α chain (Aα328) and to the fibrin α-chain/α_2 antiplasmin (Aα303) cross-linking sites. It is also within the region of the α-fibrinogen (Aα242-424) which has been suggested to enhance activation of factor XIII [4]. Such location of Thr312Ala polymorphism indicates probable influence on FXIII-dependent mechanism, essential for formation and stability of clot, what may be interesting for clinical and functional studies. Recently the A allele (312Ala) of the Thr312Ala polymorphism has been reported to be associated with post-stroke mortality among patients who have atrial fibrillation [5] and with pulmonary embolism in patients with venous thrombosis [6].

The aim of the present study was to determine the association of the Thr312Ala polymorphism in *FGA* gene with coronary artery disease.

Material and Methods

Subjects

We recruited seventy four patients with angiographically confirmed coronary artery disease (CAD), and seventy three healthy blood donors with negative family history for myocardial infarction (MI) in interview. All study individuals were white Polish Caucasians in age below 57 years. The angiographic inclusion criterion was more than 50% diameter stenosis of at least one major coronary vessel. Patients were included irrespective of concomitant risk factors for atherosclerosis such as hypertension, diabetes mellitus, body mass index (BMI) and cigarette smoking. Coronary angiography was not performed in subjects from control group and therefore in these subjects the presence of atherosclerotic coronaries cannot be excluded. The BMI was calculated as weight in kilograms divided by the square of height in meters. The study protocol was approved by the Ethics Committee of the Medical University of Silesia in Katowice and written consents from the patients were obtained.

Biochemical Analysis

All examined individuals were instructed to fast for 12–24 h before blood collection. Antecubital venous blood was collected and samples were centrifuged within 2 h of being drawn. Total serum cholesterol and triglycerides were measured by enzymatic methods (commercial Analco Kit). The coefficients of variation between the measurements for total cholesterol, and triglycerides were 1.5% and 2.0%, respectively. The plasma fibrinogen levels were measured by chronometric method using commercial bioMérieux kit.

Analysis of Polymorphisms

Genomic DNA was extracted from peripheral lymphocytes using MasterPure genomic DNA purification kit (Epicentre Technologies). The Thr312Ala polymorphism of *FGA* gene was genotyped using PCR-RFLP (restriction fragment length polymorphism) analysis. Specific primers were described previously [5] but amplification parameters were modified (initial 5 min. denaturation at 94°C, 30 cycles with 30 s at 94°C, 30 s at 64°C, 1 min. at 72°C, and final extension of 5 min. at 72°C). The PCR products were digested for 16 h at 37°C with 5 U of the *Rsa*I restriction enzyme (Fermentas) and samples were separated by 8% acrylamide gel electrophoresis and visualized by silver nitrate staining. Allele A (312Ala) gives fragments 25, 48 and 117 bp, allele T (312Thr) – 25, 39, 48 and 78 bp and genotypes were classified as TT (threonine threonine), TA (threonine alanine) and AA (alanine alanine).

Statistical Analysis

The data were analyzed using the *STATISTICA 6.0* software. Comparison of quantitative data was performed by U Mann-Whitney's test. Allele frequencies were deduced from the genotype distribution. Hardy-Weinberg equilibrium was tested

in all groups by a χ^2 test. Comparisons of genotype and allele frequencies between cases and control subjects were performed by a χ^2 test. Statistical significance was accepted at $p < 0.05$. Odds ratios (OR) as well as their 95% Cl were computed to assess the strength of the association between the presence of the polymorphic alleles and genotypes, and CAD.

Results

Clinical and biochemical parameters like mean age, BMI, total serum cholesterol and triglycerides, plasma fibrinogen level, hypertension, smoking of CAD patients and control are shown in Table 1. In the patient group there were 57% patients with hypertension, 8% with diabetes mellitus, 51% with overweight and 34% cigarette smokers whereas in control group number of smokers was higher (53%) and there were no hypertensive or diabetes mellitus individuals. Mean BMI is similar in the patients and controls. The CAD patients show increased level of total cholesterol and triglycerides. Plasma fibrinogen level is also slightly higher in patients group than in control group but we did not find correlation between plasma fibrinogen level and genotypes in both study groups.

The allele and genotype frequencies of analyzed *FGA* Thr312Ala polymorphism for study groups are reported in Table 2. The genotype frequencies among the cases and control subjects for both analyzed genes were compatible with Hardy-Weinberg equilibrium. The estimated frequencies of alleles and genotypes were compared between the CAD patients and controls.

Distribution of the Thr312Ala genotypes in patients were following: TT – 54%, TA – 42%, AA – 4% and in healthy blood donors: TT – 71%, TA – 26%, AA – 3%. Frequency of alleles among CAD patients were: T – 75%, A – 25% and in control group: T – 84%, A – 16% (Tab. 2).

Table 1. Characteristic of the study groups

Tabela 1. Charakterystyka badanych grup

	CAD patients (Chorzy na chorobę niedokrwienną serca)	Control group (Grupa kontrolna)
Number of subjects (Liczba pacjentów)	74 (%)	73 (%)
Women (Kobiety)	14 (19)	11 (15)
Men (Mężczyni)	60 (81)	62 (85)
Hypertension (Nadciśnienie tętnicze)	42 (57)	0 (0)
Smoking (Palenie papierosów)	25 (34)	39 (53)
Mean age – years ± SD (Średnia wieku – lata ± SD)	46.5 ± 6.2	35.2 ± 9.8
Mean BMI (Średnie BMI) kg/m^2 ± SD	26.6 ± 4.1	25.4 ± 3.9
Total serum cholesterol (Stężenie cholesterolu całkowitego w surowicy) mmol/l ± SD	5.64 ± 1.3*	4.91 ± 2.2
Serum TG (Stężenie trójglicerydów w surowicy) mmol/l ± SD	1.97 ± 1.0*	1.34 ± 0.5
Plasma fibrinogen (Stężenie fibrynogenu w osoczu) g/l ± SD	3.38 ± 1.4	3.09 ± 0.9

SD – standard deviation,
* Statistically significant data $p < 0.05$.

SD – odchylenie standardowe,
∗ Istotne statystycznie przy $p < 0.05$.

The A allele appeared more often in CAD patients than in healthy subjects and the difference was statistically significant ($p = 0.049$, OR = 1.78) (Tab. 3). Heterozygous carriers of A allele (TA genotype) were much more frequent in CAD patients than in

Table 2. The frequency of genotypes and alleles of the Thr312Ala polymorphism of *FGA* gene in CAD patients and control group

Tabela 2. Częstość genotypów i alleli polimorfizmu Thr312Ala w genie *FGA* u pacjentów z chorobą niedokrwienną serca i w grupie kontrolnej

Gene (Gen)		Genotypes (Genotypy)				Alleles (Allele)	
FGA		TT	TA	AA	TA+AA	T	A
CAD patients (Pacjenci z chorobą niedokrwienną serca)	n = 74 frequency	40 0.54	31 0.42	3 0.04	34 0.46	111 0.75	37 0.25
Control group (Grupa kontrolna)	n = 73 frequency	52 0.71	19 0.26	2 0.03	21 0.29	123 0.84	23 0.16

Table 3. Comparison of frequencies of A allele carriers between cases and control subjects using χ^2 test

Tebela 3. Porównanie częstości występowania nosicieli allelu A i występowania nosicieli allelu A między chorymi a osobami z grupy kontrolnej za pomocą testu χ^2

		A	TA	TA + AA
CAD patients (Pacjenci z chorobą niedokrwienną serca)	n	37	31	34
	frequency	0.25	0.42	0.46
Control group (Grupa kontrolna)	n	23	19	21
	frequency	0.16	0.26	0.29
χ^2		3.87* (p = 0.0492)	4.45* (p = 0.035)	4.63* (p = 0.031)
OR (95% CI)		1.78* (0.995–3.19)	2.12* (1.04–4.32)	2.10* (1.06–4.19)

* Statistically significant data.

* Istotne statystycznie.

control group (p = 0.035, OR = 2.12). We also found significantly higher frequency of A allele carriers (individuals with TA + AA genotypes) in CAD patients (46%) than in control group (29%) (p = 0.031, OR = 2.10) (Tab. 3). The number of A allele carriers was especially high in subgroup of male, hypertension and overweight CAD patients.

Considering gender in both study groups we found that A allele of Thr312Ala polymorphism in *FGA* gene was almost twice higher in male patients with coronary artery disease compared to male controls (28% *vs* 16%) (p = 0.022, OR = 2.06). Number of male patients possessing A allele appeared also more often in CAD group (52%) than in healthy subjects (29%) (p = 0.011, OR = 2.61) (Tab. 4). All homozygotes AA found in CAD group were men. We did not find any difference in allele and genotypes distribution between women with coronary artery disease and healthy women.

In subgroup of hypertensive CAD patients the number of A carriers was significantly higher (48%) than in control group (29%) (p = 0.042, OR = 2.25, 95%, CI 1.01 – 5.00).

Similar results were obtained for patients with overweight or obesity: 47% of A allele carriers in overweight patients *vs* 26% in control without overweight (p = 0.049, OR = 2.54, 95%CI, 0.98 – 6.57).

Discussion

In the present study we found significant association between Thr312Ala polymorphism in *FGA* gene and coronary artery disease in Polish population. We observed that A allele was significantly more frequent in whole CAD patients group and also number of carriers of A allele among patients was significantly higher than in controls. The frequency of A allele and carriers of A allele was especially high in male subgroup and in patients with hypertension and overweight. Thus known

Table 4. Comparison of frequencies of A allele and A allele carriers between male patients and male controls using χ^2 test

Tabela 4. Porównanie częstości allelu A i występowania nosicieli allelu A między pacjentami płci męskiej a osobami z grupy kontrolnej płci męskiej za pomocą testu χ^2

		A	TA + AA
Male CAD patients (Pacjenci płci męskiej z chorobą niedokrwienną serca)	n	34	31
	frequency	0.28	0.52
Male controls (Osoby płci męskiej z grupy kontrolnej)	n	20	18
	frequency	0.16	0.29
χ^2		5.27* (p = 0.0217)	6.50* (p = 0.011)
OR (95% CI)		2.06* (1.10–3.84)	2.61* (1.23–5.55)

* Statistically significant data.

* Istotne statystycznie.

risk factors may interact with this genetic variant in atherosclerosis manifestation.

Although there are many studies showing that polymorphisms in β-fibrinogen (*FGB*) gene are associated with peripheral atherosclerosis or severity and progression of coronary artery disease [7–9], we found only one study analyzing polymorphisms in *FGA* gene in CAD [10]. The authors did not find any correlation between Thr312Ala polymorphism and disease. Probably the main reason of disagreement with our results is a difference in ethnic background since citied study was conducted on non-Caucasian patients.

The Thr312Ala polymorphism was previously found to be associated with some other cardiovascular diseases. It was connected with atrial fibrillation in relation to post-stroke mortality in large group of patients with ischemic stroke [5]. The A allele carriers showed increased mortality compared with homozygotes for T allele. Carter et al. [6] reported association between Thr312Ala polymorphism and venous thrombosis. Homozygotes for A allele were significantly associated with pulmonary embolism compared to homozygotes TT.

In conclusion, the present study suggests that Thr312Ala polymorphism is associated with coronary artery disease in Polish population and carrier-state of A allele may be a risk factor for the disease.

References

[1] Henry I, Uzan G, Weil D, Nicolas H, Kaplan JC, Marguerie C, Kahn A, Junien C: The genes coding for A alpha-, B beta-, and gamma-chains of fibrinogen map to 4q2. Am J Hum Genet 1984, 36, 760–768.

[2] Yu S, Sher B, Kudryk B, Redman CM: Fibrinogen precursors. Order of assembly of fibrinogen chains. J Biol Chem 1984, 259, 10574–10581.

[3] Baumann RE, Henschen AH: Human fibrinogen polymorphic site analysis by restriction endonuclease digestion and allele-specific polymerase chain reaction amplification: identification of polymorphisms at position Aα312 and Bβ448. Blood 1993, 82, 2117–2124.

[4] Muszbek L, Adany R, Mikkola H: Novel aspects of blood coagulation factor XIII. I. Structure, distribution, activation, and function. Crit Rev Clin Lab Sci 1996, 33, 357–421.

[5] Carter AM, Catto AJ, Grant PJ: Association of the α-fibrinogen Thr312Ala polymorphism with poststroke mortality in subjects with atrial fibrillation. Circulation 1999, 99, 2423–2426.

[6] Carter AM, Catto AJ, Kohler HP, Ariëns RAS, Stickland MH, Grant PJ: α-Fibrinogen Thr312Ala polymorphism and venous thromboembolism. Blood 2000, 96, 1177–1179.

[7] Lee AJ, Fowkes FG, Lowe GD, Connor JM, Rumley A: Fibrinogen, factor VII and PAI-1 genotypes and the risk of coronary and peripheral atherosclerosis: Edinburgh Artery Study. Thromb Haemost 1999, 81, 553–560.

[8] Behague I, Poirier O, Nicaud V, Evans A, Arveiler D, Luc G, Cambou JP, Scarabin PY, Bara L, Green F, Cambien F: Beta fibrinogen gene polymorphisms are associated with plasma fibrinogen and coronary artery disease in patients with myocardial infarction. The ECTIM Study. Etude Cas Temoins sur l'Infarctus du Myocarde. Circulation 1996, 93, 440–449.

[9] de Maat MP, Kastelein JJ, Jukema JW, Zwinderman AH, Jansen H, Groenemeier B, Bruschke AV, Kluft C: –455G/A polymorphism of the beta-fibrinogen gene is associated with the progression of coronary atherosclerosis in symptomatic men: proposed role for an acute-phase reaction pattern of fibrinogen. REGRESS group. Arterioscler Thromb Vasc Biol 1998, 18, 265–271.

[10] Lee DH, Hong SH, Song J, Min WK, Kim JQ: Fibrinogen gene polymorphism in a non-Caucasian population. Clin Biochem 1999, 32, 113–117.

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