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Original research article

The FGA Thr312Ala polymorphism and risk of intracerebral haemorrhage in Polish and Greek populations

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ABSTRACT

Background and purpose: Spontaneous intracerebral haemorrhage (ICH) is the most fatal form of stroke with the highest morbidity and disability rate of all stroke types. Recent data suggest that the genetic background has a sizeable and mostly undiscovered effect on the brain haemorrhage risk. Since the coagulation system is crucial to ICH pathology, we studied the significance of the FGA Thr312Ala polymorphism in two European populations.

Materials and methods: We genotyped 550 and 224 controls as well as 261 and 242 stroke patients in Polish and Greek populations, respectively. The ICH diagnosis was confirmed by computed tomography. The FGA Thr312Ala polymorphism was analysed using real-time polymorphism chain reaction.

Results: Both crude and multivariable regression analyses showed that the studied polymorphism is a protective factor in the Polish population under the dominant and additive models of inheritance. Those results did not replicate in the Greek population. The meta-analysis of results from the Polish and the Greek populations proved that FGA Thr312Ala polymorphism affects the risk of ICH in the dominant model of inheritance.

Conclusions: The FGA Thr312Ala polymorphism affects a risk for ICH in the Polish but not in the Greek population. An advanced meta-analysis of well-designed studies with a significant number of cases might provide useful information of novel polymorphisms, including the FGA Thr312Ala polymorphism, and their role in ICH pathology.

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1. Introduction

Spontaneous intracerebral haemorrhage (ICH) is the most fatal form of stroke with the highest morbidity and disability rate of all stroke types [1]. Its prevalence is growing since the populations of developed countries are ageing and anticoagulation treatment is becoming more common in the elderly population [2,3]. Despite many clinical trials, no specific pharmacological or surgical treatment is available. In such circumstances, understanding the pathology of brain haemorrhage is crucial [4].

Intracerebral haemorrhage is caused by a rupture of a small vessel damaged either by chronic hypertension (it is more common in the deep brain structures, and hence is referred to as deep or non-lobar haemorrhage) or amyloid angiopathy (occurs usually at the border of white and grey matter, and thus is referred to as lobar haemorrhage) [5].

The most recent view on brain haemorrhage takes it to be an acute manifestation of a chronic and progressive vascular disease [6,7]. The prevalence of cerebral microbleeds (CMBs) has been found to be associated with ICH [8], as well to be a predictor of ICH [9]. Thus, haemorrhagic stroke is assumed to be a clinical manifestation of the evolution of one of many microbleeds to an extended haematoma. The process depends on the size and type of vascular damage, local inflammation response, as well as the properties and characteristics of the coagulation system.

Apart from the well-established ICH risk factors, epidemiological data suggest that the genetic background has a sizeable and mostly undiscovered effect on the brain haemorrhage risk [4]. Since the main goal of the haemostatic system is to maintain the balance between bleeding and coagulation, the genetic mutations of coagulation factors that alter its functioning are natural candidates for the ICH risk factors.

The *FGA* Thr312Ala (A > G) polymorphism occurs in the region of fibrinogen, which is crucial to factor XIII-induced cross-linking of fibrin fibres and causes amino-acid substitution. The clot forming process includes a series of FXIII-dependent protein cleavages and new chemical bonds generation. The amino-acid substitution changes the structure of a mature protein and lowers its affinity for degrading enzymes, which consequently leads to increased clot's resistance to thrombolysis and risk of intravascular embolisation [10]. Hence the *FGA* Thr312Ala polymorphism has been proved a risk factor for venous thromboembolism [11] and chronic thromboembolic pulmonary hypertension [12].

Moreover, we previously showed that fibrin clot properties play a significant role in the acute phase of ICH [13]. Since in ICH, in contrast to intravascular coagulation in the above-mentioned conditions, uncontrolled bleeding is a major pathology, we hypothesize that the analyzed polymorphism might have a protective effect on brain haemorrhage.

The aim of that paper is to analyze the correlation between the ICH prevalence and the polymorphism of the fibrinogen alpha chain gene *FGA* Thr312Ala. As it is widely acknowledged that genetic case-control studies should be replicated in ethnically different populations, we decided do compare the results obtained from the Polish population with those received from the Greek population. Many studies have shown that there are major differences in the genetic structure of the southern (e.g. Greek, Spanish, Italian) European populations and those with northern European ancestry (including the Polish populations) [14,15].

2. Materials and methods

Two hundred and sixty-one patients presenting with haemorrhagic stroke, admitted to the Stroke Unit, Department of Neurology, University Hospital in Krakow, Poland, between 1999 and 2009 were enrolled into the study. The control group consisted of 550 individuals without stroke, matched for sex and age. All the patients and controls were Caucasians and of Polish origin.

Moreover, 242 patients with haemorrhagic stroke treated in the General University Hospital of Larissa and 224 healthy controls recruited from the Greek populations living in the area of Larissa in the Central Greece, matched for sex and age were analyzed and genotyped to replicate the study results [16].

The ICH diagnosis was confirmed by computed tomography (CT). Computed tomography angiography or magnetic resonance imaging was used for identifying or excluding secondary causes of haemorrhage, including arteriovenous malformations, tumours, and cerebral vein thrombosis.

Standardised questionnaire on demographics, medical history and vascular risk factors, including hypertension, ischaemic heart disease, myocardial infarction, atrial fibrillation, hypercholesterolaemia, diabetes mellitus, alcohol intake and smoking was completed by each of the participants, both from the Polish and from the Greek populations.

The healthy controls were recruited from the hospital staff families, consecutive spouses of the patients, or were volunteers from the community. They had no medical history of neurological disorders and were matched according to the International Stroke Genetic Consortium (ISGC) criteria [17]. All the subjects, both in the Polish and Greek cohorts, gave an informed consent. The study project was approved by the Jagiellonian University Medical College and the University Hospital ethics committee in Poland and by the Univeristy Research Committee of the University of Larissa.

Genomic DNA was extracted from peripheral blood using a commercially available kit from QIAGEN® (QIAmp, Blood Mini Kit). The *FGA* Thr312Ala polymorphism was determined by the real time polymerase chain reaction (RT PCR) using TaqMan 7900 device (by Applied Biosystems, Carlsbad, California). The genotyping results were analyzed using Applied Biosytems software (Sequence Detection System, SDS). In ambiguous cases, we verified the results by the standard polymerase chain reaction (PCR) with enzymatic digestion and subsequent gel electrophoresis.

To perform a genetic analysis, we used 'Statistica' and 'SAS Genetics' software. To conduct a meta-analysis of the two studied populations, we applied the two-value binary logic model optimised by the Newton–Raphson method. The

Table 1 – The demographic characteristics and vascular risk factors profile in patients with intracerebral haemorrhage (ICH) and control group in Polish and Greek populations (model 1 – crude analysis of risk factors and risk of ICH; model 2 – analysis of risk factors and risk of ICH after adjustment for age and sex).

	ICH patients		Controls		OR (95% CI) (p)[a]		OR (95% CI) (p)[b]	
	Polish population	Greek population	Polish population	Greek population	Polish population	Greek population	Polish population	Greek population
Age	65.0 (13.9)	64.1 (13.0)	57.1 (17.1)	64.2 (12.8)	1.03 (1.02–1.04) (<0.01)	1.0 1.0–1.0 (0.94)	1.03 1.02–1.04 (<0.01)	1.0 1.0–1.0 (0.93)
Gender (male)	141 (51.6%)	159 (63.6%)	340 (44.9%)	163 (65.2%)	1.3 1.0–1.7 (0.05)	0.9 0.6–1.3 (0.71)	1.5 1.1–2.0 (<0.01)	0.9 0.6–1.3 (0.71)
Hyper-tension	216 (80.6%)	199 (79.6%)	297 (39.7%)	113 (45.2%)	6.3 4.5–8.8 (<0.01)	4.7 3.2–7.0 (<0.01)	5.3 3.7–7.5 (<0.01)	5.7 3.7–8.7 (<0.01)
Ischaemic heart disease	82 (30.7%)	31 (12.4%)	165 (22.1%)	32 (12.8%)	1.6 1.1–2.1 (<0.01)	1.0 0.6–1.6 (0.89)	1.0 0.7–1.4 (0.99)	1.0 0.6–1.7 (0.88)
Diabetes	48 (18.3%)	32 (12.8%)	72 (9.6%)	35 (14.0%)	2.1 1.4–3.1 (<0.01)	0.9 0.5–1.5 (0.69)	1.6 1.1–2.4 (0.02)	0.9 0.5–1.5 (0.712)
Smoking	67 (28.2%)	58 (23.3%)	178 (23.8%)	37 (14.8%)	1.3 0.9–1.7 (0.18)	1.7 1.1–2.7 (0.02)	1.5 1.0–2.1 (0.03)	1.8 1.1–2.8 (0.02)
Alcohol	19 (7.9%)	72 (28.8%)	70 (9.4%)	62 (24.8%)	0.8 (0.5–1.4) (0.50)	1.2 0.8–1.8 (0.31)	0.9 (0.5–1.6) (0.69)	1.2 0.8–1.9 (0.28)
Hyper-cholesterolaemia	101 (39.0%)	83 (33.2%)	203 (29.9%)	77 (30.8%)	1.5 (1.1–2.0) (0.01)	1.1 0.8–1.6 (0.57)	1.2 (0.9–1.7) (0.18)	1.1 0.8–1.6 (0.56)

OR, odds ratio; CI, confidence interval.
[a] χ^2-test – one degree of freedom, Table 2 × 2.
[b] Logistic analysis after adjustment for sex and age.

Table 2 – *FGA* Thr312Ala (A > G) (rs6050) polymorphism at the 4 chromosome and intracerebral haemorrhage (ICH) risk in Polish and Greek populations.

	ICH (lobar and deep)				ICH (deep)			
	N (%)	*N* (%)	OR (95% CI) (*p*)	OR (95% CI) (*p*)	*N* (%)	*N* (%)	OR (95% CI) (*p*)	OR (95% CI) (*p*)
Polish population								
Genotypes								
AA	167 (64.0%)	326 (59.3%)	2.9 (1.2–7.0) (0.01)[a]	6.9 (1.5–30.9) (0.01)[b]	102 (65.4%)	326 (59.2%)	2.1 (0.8–5.3) (0.13)[a]	3.0 (0.9–10.4) (0.09)[b]
AG	88 (33.7%)	189 (34.4%)	1.2 (0.9–1.7) (0.20)[c]	1.4 (1.0–2.1) (0.07)[d]	49 (31.4%)	189 (34.4%)	1.3 (0.9–1.9) (0.17)[c]	1.4 (1.0–2.2) (0.08)[d]
GG	6 (2.3%)	35 (6.4%)	0.8 (0.6–1.0) (0.05)[e]	0.7 (0.5–0.9) (0.01)[f]	5 (3.2%)	35 (6.4%)	0.8 (0.6–1.0) (0.09)[e]	0.7 (0.5–1.0) (0.04)[f]
Allele								
A	422 (80.8%)	841 (76.5%)	0.8 (0.6–1.0) (0.05)[‡]	0.7 (0.5–0.9) (0.01)[‡‡]	253 (81.1%)	841 (76.5%)	0.8 (0.6–1.0) (0.08)[‡]	0.7 (0.5–1.0) (0.04)[‡‡]
G	100 (19.2%)	259 (23.5%)			59 (18.9%)	259 (23.5%)		
Greek population								
Genotypes								
AA	142 (58.7%)	145 (64.7%)	1.2 (0.5–2.8) (0.69)[a]	1.4 (0.6–3.5) (0.46)[b]	96 (59.6%)	145 (64.7%)	1.1 (0.4–2.7) (0.87)[a]	1.5 (0.5–4.0) (0.46)[b]
AG	89 (36.8%)	67 (29.9%)	0.8 (0.5–1.1) (0.18)[c]	0.8 (0.5–1.2) (0.28)[d]	57 (35.4%)	67 (29.9%)	0.8 (0.5–1.2) (0.31)[c]	0.9 (0.6–1.4) (0.60)[d]
GG	11 (4.5%)	12 (5.4%)	1.2 (0.9–1.6) (0.34)[e]	1.1 (0.8–1.6) (0.54)[f]	8 (5.0%)	12 (5.4%)	1.1 (0.8–1.6) (0.44)[e]	1.0 (0.7–1.5) (0.88)[f]
Allele								
A	373 (77.1%)	357 (79.7%)	1.2 (0.9–1.6) (0.33)[‡]	1.1 (0.8–1.6) (0.54)[‡‡]	249 (77.3%)	357 (79.7%)	1.2 (0.8–1.6) (0.43)[‡]	1.0 (0.7–1.5) (0.88)[‡‡]
G	111 (22.9%)	91 (20.3%)			73 (22.7%)	91 (20.3%)		

OR, odds ratio; CI, confidence interval.

[a] χ^2 test (one degree of freedom, Table 2 × 2), performed for dominant effect of A allele (AA + AG vs. GG) in both analyzed groups (patients presented with ICH and control group).

[b] Logistic regression model testing dominant effect of A allele (AA + AG vs. GG) on ICH risk after adjustment for sex, age and vascular risk factors (hypertension, diabetes, smoking, hypercholesterolaemia) (patients presented with ICH and control group).

[c] χ^2 test (one degree of freedom, Table 2 × 2), performed for recessive effect of A allele (AA vs. AG + GG) in both analyzed groups (patients presented with ICH and control group).

[d] Logistic regression model testing recessive affect of A allele (AA vs. AG + GG) on the ICH risk after adjustment for sex, age and vascular risk factors (hypertension, diabetes, smoking, hypercholesterolaemia) (patients presented with ICH and control group).

[e] Additive model applied by trend test (*Cochran–Armitage trend test*) in both analyzed groups (patients presented with ICH and control group).

[f] Logistic regression model testing additive affect (AA vs. AG vs. GG) on ICH risk after adjustment for sex, age and vascular risk factors (hypertension, diabetes, smoking, hypercholesterolaemia) (patients presented with ICH and control group).

[‡] χ^2 test (one degree of freedom, Table 2 × 2), performed on A and G alleles in both analyzed groups (patients presented with ICH and control group).

[‡‡] χ^2 test (one degree of freedom, Table 2 × 2), performed on A and G alleles in both analyzed groups (patients presented with ICH and control group) after adjustment for sex and age.

analysis of the studied groups was performed using χ^2-test or Student's *t*-test. The Hardy–Weinberg equilibrium was tested by χ^2-test. The association of the *FGA* Thr312Ala polymorphism with ICH was tested using the multivariable logistic regression under dominant (AA + AG vs. GG), recessive (AA vs. AG + GG) or additive (AA vs. AG vs. GG) models of inheritance. A *p*-value less than 0.05 was considered as significant.

3. Results

We genotyped 261 patients with haemorrhagic stroke and 550 controls from the Polish population as well as 242 patients and 224 controls from the Greek population. There was no deviation from the Hardy–Weinberg equilibrium regarding the *FGA* Thr312Ala polymorphism in each of the studied groups ($p > 0.05$). The demographic characteristics and vascular risk factors profile for the Polish and Greek populations are summarised in Table 1.

Out of 261 patients who were successfully genotyped in the Polish population, 60% ($n = 156$) presented with deep haemorrhage and 39% ($n = 102$) with lobar haemorrhage. Likewise, in the Greek population 67% ($n = 161$) of the patients manifested deep haemorrhage and 33% ($n = 81$) lobar haemorrhage. The alleles and genotypes distribution was significantly different between the patients with ICH and controls in the Polish population (patients: AA – 64.0% ($n = 167$), AG – 33.7% ($n = 88$), GG – 2.3% ($n = 6$), A – 80.8% ($n = 422$); controls: AA – 59.3% ($n = 326$), AG – 34.4% ($n = 189$), GG – 6.4% ($n = 35$); A – 76.5% ($n = 841$); χ^2 test, $p < 0.05$) but not in the Greek population (patients: AA – 58.7% ($n = 142$), AG – 36.8% ($n = 89$), GG – 4.5% ($n = 11$), A – 77.1% ($n = 373$); controls: AA – 64.7% ($n = 145$), AG – 29.9% ($n = 67$), GG – 5.4% ($n = 12$); A – 79.7% ($n = 357$); χ^2 test, $p > 0.05$) (Table 2). Similarly, we found a significant difference in the alleles but not in the genotypes distribution in patients with deep haemorrhage in the studied Polish population (Table 2).

Moreover, the multivariable logistic regression analysis, both crude and adjusted for vascular risk factors, showed the significance of the *FGA* Thr312Ala polymorphism as a protective factor for haemorrhagic stroke, under the dominant and additive models of inheritance (Table 2).

Those results did not replicate in the Greek population. The analysis of the combined results proved the effect of the analyzed polymorphism on ICH in the dominant model of inheritance (Table 3).

4. Discussion

We found that the *FGA* Thr312Ala is associated with ICH in the Polish but not in the Greek population. Multivariable logistic regression adjusted for vascular risk factors revealed that the AA (Ala) genotype of the studied polymorphism has a higher prevalence in the Polish population of patients with brain haemorrhage under the dominant and additive model of inheritance. In the group of patients with deep haemorrhage in the Polish population, the A allele was significantly more frequent than in the control population. The analysis of the combined data showed that the AA genotype is significantly correlated with a higher prevalence of ICH in the two analyzed populations.

No literature analysing the *FGA* Thr312Ala polymorphism as a risk factor for ICH has been published so far.

As we mentioned in the introduction, we assumed that genetic variations that influence the clotting process and therefore alter the enlargement of small brain microbleeds might be a proper candidate for investigation of novel ICH-related genes.

Moreover, we showed in the previous publication that in acute ICH, some properties of fibrin clot are changed, e.g. the time of clot lysis, which supports the hypothesis that coagulation and clotting are crucial to the process of small bleeding evolving to haematoma [13]. Other factors (clot compaction, maximum rate of D-dimer release in the lysis assay) correlate with the haematoma volume or neurological deficit examined by the National Institutes of Health Stroke Scale (NIHSS).

A question might be posed if we should analyze the association of the studied polymorphism and ICH prevalence or whether we should try to find a correlation with the clinical markers of ICH progress such as the haematoma volume change in time, or clinical assessment.

Another problem is that the obtained results do not replicate in the Greek population. We did not confirm any trends in the Greek group of patients with ICH. The reason might be a small and inadequate number of the analyzed cases and controls, which suggests a need for a study with a higher power. What is more, substantial differences in the demography of the patient and control groups were observed in the Polish population (Table 1). This might be one of the reasons why the results in the Greek cohort did not replicate. Besides, in the previous co-authored publication, we did not confirm

Table 3 – The combined analysis of *FGA* Thr312Ala (A > G) (rs6050) polymorphism in Polish and Greek population.

Polymorphism	Polish population OR (95% CI) (*p*)	Greek population OR (95% CI) (*p*)	Analysis of combined results[a] OR (95% CI) (*p*)
FGA Thr312Ala (A > G) (dominant model, adjusted for age, sex, hypertension and smoking)	6.8 (1.5–30.0) (0.01)	1.4 (0.6–3.5) (0.46)	2.3 (1.1–4.8) (0.03)
FGA Thr312Ala (A > G) (additive model, adjusted for age, sex, hypertension and smoking)	0.7 (0.5–0.9) (0.01)	1.1 (0.8–1.5) (0.57)	0.9 (0.7–1.1) (0.36)

OR, odds ratio; CI, confidence interval.
[a] Two-value binary logic model optimised by the Newton–Raphson method.

the common mechanisms of haemorrhagic stroke pathology in those two populations [18]. It might suggest that the genetic background of ICH varies within the specific European subpopulations.

Thorough understanding of coagulation process seems to be the most recent and promising method for specific ICH prevention. Anticoagulant therapy, adjusted to the genetic profile, might significantly reduce the ratio of undesirable complications, including ICH. Henceforth, novel ICH-related genes identification is crucial to finding new ICH prevention strategies.

5. Conclusions

1. The *FGA* Thr312Ala polymorphism affects a risk for ICH in the Polish but not in the Greek population.
2. An advanced meta-analysis of well-designed studies with a significant number of cases might provide useful information of novel polymorphisms, including the *FGA* Thr312Ala polymorphism, and their role in ICH pathology.

Conflict of interest

None declared.

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Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; Uniform Requirements for manuscripts submitted to Biomedical journals.

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