Association of polymorphisms of platelet receptors GPIa (807C>T), GPVI (13254T>C), and P2Y12 (34C>T and H1/H2 haplotype) with increased risk of periprocedural bleeding in patients undergoing coronary angiography/ percutaneous coronary intervention

Magdalena Sionova[1], Peter Blasko[2], Stepan Jirous[3], David Vindis[4], Richard Rokyta[3], Zuzana Motovska[1]

[1]Third Faculty of Medicine, Faculty Hospital, Kralovske Vinohrady, Czech Republic
[2]Faculty of Medicine, University Hospital, Ostrava, Czech Republic
[3]Faculty of Medicine, University Hospital, Pilsen, Czech Republic
[4]Faculty of Medicine and Dentistry, University Hospital, Olomouc, Czech Republic

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Abstract

Introduction: Periprocedural bleeding related to coronary angiography (CAG) or percutaneous coronary intervention (PCI) is associated with worse prognosis. Determining genetic variations associated with increased bleeding risk may help to identify high-risk patients.

Aim: To analyse the association between single nucleotide polymorphisms (SNPs) of crucial haemostatic platelet receptors (GPIa, GPVI, P2Y12) and the risk of periprocedural bleeding complications related to CAG/PCI.

Material and methods: The population consisted of 73 patients with ischaemic heart disease who developed bleeding complications within 30 days after CAG/PCI and 331 patients without bleeding. The frequency of SNPs of GPIa 807C/T, GPVI 13254T/C, P2Y12 32C/T, and P2Y12 H1/H2 haplotype was analysed using polymerase chain reaction (PCR) hybridization methods.

Results: The prevalence of variant alleles GPIa 807T, GPVI 13254C, P2Y12 34T, and P2Y12 H2 haplotype in the total study population was 56.7%, 20.3%, 56.2%, and 24.3%, respectively. The presence of variant alleles was not related to increased risk of periprocedural bleeding: GPIa 807C/T (OR = 1.29, 95% CI: 0.75–2.24, p = 0.334), GPVI 12354T/C (OR = 0.82, 95% CI: 0.40–1.64, p = 0.551), P2Y12 34C/T (OR = 0.71, 95% CI: 0.42–1.22, p = 0.189), P2Y12 H1/H2 haplotype (OR = 0.69, 95% CI: 0.35–1.36, p = 0.258). The frequency of the homozygous form of P2Y12 H2 haplotype was higher in the group of patients who developed bleeding (OR = 2.79, 95% CI: 0.51–13.77, p = 0.161).

Conclusions: No significant association of the SNPs of GPIa 807C/T, GPVI 13254T/C, P2Y12 32C/T, and P2Y12 H1/H2 haplotype with increased risk of periprocedural bleeding was found in patients with ischaemic heart disease undergoing CAG/PCI.

Key words: ischaemic heart disease, periprocedural bleeding, platelet receptors, single nucleotide polymorphism.

Introduction

Periprocedural bleeding is the most common complication related to coronary angiography (CAG) or percutaneous coronary intervention (PCI) in patients with ischaemic heart disease, and it is associated with a worse short-term as well as long-term prognosis [1–4]. With the advances in techniques of cardiac catheterization and coronary artery interventions and with the development of more effective antiplatelet and anticoagulant drugs, the efforts to reduce the occurrence of periprocedural bleeding are gaining interest [5]. A series of avoidance bleeding strategies has been identified (radial access, smaller sheath size, optimal choice and dose of antiplatelet and anticoagulant drugs, other special measures) [6]. These measures are most effective when used in patients at the highest risk of periprocedural bleeding [7]. Thus it is crucial to identify those high-risk patients in whom the individual use of avoidance bleeding strate-

Corresponding author:
Magdalena Sionova, Third Faculty of Medicine, Faculty Hospital, Kralovske Vinohrady, 50 Srobarova St, Praha 10, 10034 Prague, Czech Republic, phone: 607963728, e-mail: magda.doktorova@post.cz
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gies may reduce the risk of bleeding and so may improve their prognosis.

Mapping and identifying the genetic basis of human diseases and their risk factors is becoming considerably important in the current trend of individualization of patient care which is applied across all medical fields [8]. An association with increased risk of coronary heart disease or ischaemic stroke development has been demonstrated for 60 genetic loci so far [9]. While platelets and their regulatory proteins play a fundamental role in the process of atherothrombosis as well as haemostasis in patients with ischaemic heart disease, single nucleotide polymorphisms (SNPs) of key platelet proteins may lead to bleeding tendencies. Based on detailed knowledge of the haemostasis pathways the decisive platelet receptors responsible for adhesion, activation and aggregation and their SNPs were selected for the analysis (GPIa 807C/T, GPVI 12354T/C, P2Y12 H1/H2 haplotype and P2Y12 32C/T) [10].

Aim

Aim of the study was to analyse the association between single nucleotide polymorphisms (SNPs) of crucial haemostatic platelet receptors (GPIa, GPVI, P2Y12) and the risk of periprocedural bleeding complications related to CAG/PCI.

Material and methods

Patient population and bleeding definition

The study was designed as a case-control study. Seventy-three patients with an acute or a chronic form of ischaemic heart disease who developed any clinically significant periprocedural bleeding complication within 30 days after a diagnostic cardiac catheterization (CAG) or a percutaneous coronary intervention (PCI) were enrolled between the years 2010 and 2014 from five catheterization centres in the Czech Republic (University Hospital Kralovske Vinohrady, University Hospital Ostrava, University Hospital Pilsen, University Hospital Olomouc). The Ethics Committees of participating institutions approved the study protocol. The patients were included in the study after signing informed consent for participation. No exclusion criteria were applied for the study participation. The control group consisted of 331 patients from the genetic subanalysis of the PRAGUE-8 trial who had not developed any bleeding complication within 30 days after the invasive procedure (CAG/PCI).

Periprocedural bleeding was defined as: i) an access site subcutaneous haematoma with a diameter larger than 5 cm; ii) a type of access site bleeding other than subcutaneous (intramuscular, retroperitoneal); iii) gastrointestinal bleeding, bleeding from the urinary tract or obvious bleeding from any other source (haemoptysis, gynaecological bleeding, epistaxis, etc.); iv) pericardial bleeding; v) intracranial bleeding.

Basic demographic data, medical history, cardiovascular risk factors, major comorbidities, clinical condition and laboratory findings were recorded in all patients. The medical history was obtained from the patient's medical documentation or personal interview. The clinical evaluation was performed during admission to the hospital and then any relevant changes of the clinical status were noted during the hospitalization. A blood count was obtained from each patient with a special emphasis on the haematocrit and the haemoglobin level. Kidney function was monitored by creatinine clearance (estimated by Cockcroft-Gault formula). All bleeding complications that occurred were precisely registered from multiple perspectives (localization, size, affecting structures, clinical impact, drop of haemoglobin level). The localization of each bleeding was observed using various imaging techniques when needed and applicable (computed tomography – CT, ultrasound – US).

Genetic analysis

A sample of peripheral venous blood was taken from each patient after signing the informed consent. The presence of SNPs of key platelet receptors GPIa (807C>T, rs1126643), GPVI (13254T>C, rs1613662), and P2Y12 (34C>T, rs6785930 and H1/H2 haplotype, rs2046934) was investigated. The testing was based on polymerase chain reaction (PCR) amplification of specific DNA sequences coding selected proteins. The DNA was extracted from 200 µl of peripheral blood using a standard spin-column procedure and a JetQuick DNA isolation kit (GENOMED GmbH, Loehne, Germany). The sequences of PCR amplicons were analysed using denaturing capillary electrophoresis (DCE) that could reveal the presence of any variation within the target region. Using this method, fluorescently labelled PCR amplicons were separated into homo- and heteroduplexes at a precise temperature on a standard DNA sequencer (ABI PRISM 3100, Applied Biosystems, Foster City, Calif., USA). This technique determines the relative fraction of mutated alleles. All detected DNA variations were confirmed by direct sequencing using the same DNA sequencer.

Statistical analysis

The absolute frequencies with relative abundances in percentage were counted for categorical data and an assay based on χ^2 (chi-square) testing was used for comparison. For continuous variables, arithmetic means and standard deviations were calculated and compared using the non-parametric Mann-Whitney test. The frequencies of SNPs were investigated. Odds ratios and corresponding 95% confidence intervals were used to measure the association between the variables. All statistical tests were assessed for the significance level of $p < 0.05$. SPSS statistical software version 13.0 (SPSS Inc., Chicago, Illinois) was used for statistical analysis. All statistical anal-

yses were performed by a statistician with experience in biomedical statistics.

Results

The total population consisted of 404 patients. The mean age was 66.8 ±9.57 years. 60.1% of patients were male, average body mass index (BMI) was 28.7 ±4.37 kg/m^2, 18.4% of patients were smokers, 66.1% of patients had arterial hypertension, 34.3% had a history of ischaemic heart disease, 61.8% suffered from dyslipidaemia, 27.5% had diabetes and 7.2% of patients had previous stroke/transient ischemic attack (TIA). Univariate analysis showed that the patients who developed periprocedural bleeding complication were older (p = 0.002), had lower average BMI (p = 0.017), more often had arterial hypertension (p = 0.037), renal insufficiency (defined as clearance creatinine < 60 ml/min) (p = 0.003), and a history of previous bleeding (p = 0.017), a higher leucocyte count (p = 0.002), lower admission haemoglobin and haematocrit level (p = 0.001) and lower international normalised ratio (INR; p = 0.001). Procedure-related data showed that the patients with periprocedural bleeding presented more often with an acute coronary syndrome (p = 0.001) or underwent PCI (p = 0.001). Also hospital stay was significantly longer when bleeding occurred (p = 0.001). Details and other characteristics are displayed in Table I.

Table I. Baseline characteristics

Parameter	Patients without bleeding (n = 331)	Patients with bleeding (n = 73)	P-value*
Age, mean ± SD [years]	66.08 ±9.330	70.21 ±9.980	0.002
Male, *n* (%)	204 (61.8)	38 (52.1)	0.123
Height, mean ± SD [cm]	169.81 ±8.699	168.57 ±9.223	0.308
Weight, mean ± SD [kg]	83.59 ±14.503	79.89 ±17.519	0.027
BMI, mean ± SD [kg/m^2]	28.941 ±4.28	27.653 ±4.63	0.017
BMI < 18.5 kg/m^2, *n* (%)	3 (0.9)	1 (1.4)	0.717
BMI > 30 kg/m^2, *n* (%)	116 (35.3)	16 (22.9)	0.030
Cigarette smoking, *n* (%)	58 (17.6)	16 (22.2)	0.357
History of ischaemic heart disease, *n* (%)	114 (35.1)	24 (32.9)	0.721
Arterial hypertension, *n* (%)	211 (63.9)	56 (76.7)	0.037
Diabetes mellitus, *n* (%)	94 (28.6)	17 (23.9)	0.430
Dyslipidaemia, *n* (%)	211 (63.9)	38 (52.1)	0.059
Stroke/TIA, *n* (%)	21 (6.3)	8 (11.0)	0.169
Peripheral artery disease, *n* (%)	28 (8.5)	8 (13.3)	0.236
Pulmonary embolism/ DVT, *n* (%)	12 (4.1)	4 (5.5)	0.613
Atrial fibrillation, *n* (%)	38 (13.1)	13 (17.8)	0.296
Renal insufficiency (cl. cr < 60 ml/min), *n* (%)	67 (20.6)	26 (37.1)	0.003
History of bleeding, *n* (%)	5 (1.7)	5 (6.8)	0.017
History of cancer, *n* (%)	12 (4.1)	6 (8.2)	0.149
Ejection fraction, mean ± SD	53.65 ±17.545	52.09 ±12.918	0.025
Hospital stay, mean ± SD [days]	4.38 ±2.729	8.59 ±6.166	0.001
Leukocytes, mean ± SD [× 10^9/l]	7.751 ±2.326	9.282 ±3.582	0.002
Haematocrit, mean ± SD (%)	41.637 ±6.942	34.851 ±13.747	0.001
Haemoglobin, mean ± SD [mmol/l]	140.635 ±14.551	135.180 ±18.768	0.020
Platelet count, mean ± SD [× 10^9/l]	240.472 ±66.512	235.548 ±83.146	0.152
INR, mean ± SD	1.052 ±0.098	1.165 ±0.336	0.001
Acute coronary syndrome, *n* (%)	56 (16.9)	45 (61.6)	0.001
PCI, *n* (%)	89 (27)	48 (65.8)	0.001

*BMI – body mass index, DVT – deep vein thrombosis, INR – international normalized ratio, PCI – percutaneous coronary intervention, SD – standard deviation, TIA – transitory ischaemic attack. *p-value – without bleeding vs. with bleeding.*

The frequency of bleeding according to the type is displayed in Table II. The majority of bleeding events occurred at the access site (82.2%).

Table III shows frequencies of selected SNPs in the total population. The most frequent variant allele (together in its homozygous and heterozygous form) was the 807T allele of the GPIa gene, which occurred in 56.7%, and the 34T allele of the P2Y12 gene, which occurred in 56.2% of studied individuals. These polymorphisms also represented the highest number of the homozygous form alone (12.4% and 10.2%, respectively). H2 haplotype of the P2Y12 gene occurred in 24.3%, and 12354C polymorphism of the GPVI gene in 20.3%. The rate of homozygous forms of P2Y12 H2/H2 and GPVI 12354CC were low (2% and 1.5%).

The relation between the studied SNPs and the development of periprocedural bleeding after the CAG/PCI in the whole patient population is displayed in Table III. No statistically significant association was found. The analysis of the association between the risk of periprocedural bleeding and the homozygous forms (which are assumed to have stronger phenotypic expression) showed higher prevalence (but without significance) of P2Y12 H2/H2 haplotype in patients who developed bleeding (odds ratio (OR) = 2.79, 95% conflidence interval (CI): 0.51–13.77, p = 0.161) (Table IV).

As patients undergoing diagnostic CAG are usually stable, are assumed to have a different level of thrombotic risk, and do not receive such aggressive anticoagulant and antiplatelet therapy, the risk of bleeding is generally lower than in patients with PCI. That is the reason we evaluated the association between the SNPs and the risk of periprocedural bleeding in both those groups separately. The complete results are shown in Table V. We did not find any significant association between the SNPs and increased risk of bleeding even in the CAG or the PCI group.

The distribution of SNPs depending on gender is displayed in Table VI. We verified that the occurrence of neither of the polymorphisms is sex-linked.

Discussion

Genetic testing is becoming a part of routine clinical practice in pharmacogenetics (individualized drug therapy according to the genetic variabilities for clopidogrel and warfarin) and is also extending into preventive measures [11]. A higher level of LDL cholesterol and accelerated atherosclerosis were reported in carriers of the gain of function (GOF) allele for the PSCK9 gene (proprotein convertase subtilisin/kexin type 9), the enzyme responsible for low density lipoprotein (LDL) receptor degradation on the cell surface [12]. Currently, an antibody against PCSK9 (evolocumab), which may effectively reduce the LDL cholesterol level by up to 40–50%, has been introduced into clinical practice. A higher risk of bleeding associated with certain genetic variability is proved for the CYP2C19*17 polymorphism in patients treated with clopidogrel [13–15]. As periprocedural bleeding may be preventable by using a series of known bleeding avoidance strategies with the most significant benefit in high-risk patients [6], knowledge of the genetic polymorphisms that are associated with higher risk of periprocedural bleeding may lead to more precise identification of those high-risk patients.

Table II. Number of bleeding complications according to type

Type of bleeding	Number of bleeding complications according to type
Access site haematoma	60 (82.2%)
Retroperitoneal bleeding	2 (2.7%)
Gastrointestinal bleeding	1 (1.4%)
Urinary tract bleeding	1 (1.4%)
Epistaxis	2 (2.7%)
Pericardial bleeding	3 (4.1%)
Others (haemoptysis, gynaecological bleeding, etc.)	4 (5.5%)

Table III. Number of allele carriers in total study population

Identification of single nucleotide polymorphism	Allele type	Total study population (n = 404) n (%)
GPIa (807C/T)	HET + HOM	228 (56.7)
	HET (CT)	178 (44.3)
	HOM (TT)	50 (12.4)
	Wild type (CC)	174 (43.3)
GPVI (13254T/C)	HET + HOM	82 (20.3)
	HET (TC)	76 (18.8)
	HOM (CC)	6 (1.5)
	Wild type (TT)	322 (79.7)
P2Y12 (H1/H2 haplotype)	HET + HOM	98 (24.2)
	HET (H1/H2)	90 (22.3)
	HOM (H2/H2)	8 (2.0)
	Wild type (H1/H1)	305 (75.5)
	N/A	1 (0.3)
P2Y12 (34C/T)	HET + HOM	226 (55.9)
	HET (CT)	185 (45.8)
	HOM (TT)	41 (10.1)
	Wild type (CC)	176 (43.6)
	N/A	2 (0.5)

GP – glycoprotein, HET – variant allele in heterozygous form, HOM – variant allele in homozygous form.

Table IV. Association between the single nucleotide polymorphisms and the risk of bleeding in patients undergoing coronary angiography or percutaneous coronary intervention

Identification of single nucleotide polymorphism	Allele type	Patients without bleeding *n* = 331 *n* (%)	Patients with bleeding *n* = 73 *n* (%)	Risk of bleeding (OR)	95% confidence interval	*P*-value
GPIa (807C/T)	HET + HOM	183 (55.5)	45 (61.6)	1.29	0.75–2.24	0.334
	HOM	40 (12.2)	10 (13.7)	1.15	0.51–2.54	0.718
GPVI (13254T/C)	HET + HOM	69 (20.9)	13 (17.8)	0.82	0.40–1.64	0.551
	HOM	5 (1.5)	1 (1.4)	0.91	n/a	0.928
P2Y12 (H1/H2 haplotype)	HET + HOM	84 (25.5)	14 (19.2)	0.69	0.35–1.36	0.258
	HOM	5 (1.5)	3 (4.1)	2.79	0.51–13.77	0.161
P2Y12 (34C/T)	HET + HOM	190 (58.7)	37 (50.7)	0.71	0.42–1.22	0.189
	HOM	36 (10.9)	5 (6.8)	0.60	0.20–1.67	0.296

GP – glycoprotein, HET – variant allele in heterozygous form, HOM – variant allele in homozygous form, OR – odds ratio.

Table V. Association between single nucleotide polymorphisms and risk of bleeding separately in patients undergoing percutaneous coronary intervention or coronary angiography

Identification of single nucleotide polymorphism	Allele type	Patients without bleeding *n* (%)	Patients with bleeding *n* (%)	Risk of bleeding (OR)	95% confidence interval	*P*-value
PCI group		***n* = 89**	***n* = 48**			
P2Y12 (H1/H2 haplotype)	HET + HOM	22 (24.7)	9 (18.8)	0.70	0.27–1.81	0.426
P2Y12 (34C/T)	HET + HOM	51 (57.3)	23 (47.9)	0.69	0.32–1.47	0.293
GPVI (13254C/T)	HET + HOM	19 (21.3)	10 (20.8)	0.97	0.37–2.48	0.943
GPIa (807C/T)	HET + HOM	50 (56.2)	32 (66.7)	1.56	0.71–3.47	0.232
CAG group		***n* = 242**	***n* = 25**			
P2Y12 (H1/H2 haplotype)	HET + HOM	62 (25.8)	5 (20.0)	0.72	0.23–2.14	0.523
P2Y12 (34C/T)	HET + HOM	138 (57.7)	13 (52.0)	0.79	0.32–1.95	0.581
GPVI (13254T/C)	HET + HOM	50 (20.8)	3 (12.0)	0.52	0.12–1.93	0.293
COX-1 (–842A/G)	HET + HOM	26 (10.8)	2 (8.0)	0.72	0.11–3.43	0.665
COX-1 (50C/T)	HET + HOM	26 (10.9)	2 (8.2)	0.74	0.11–3.55	0.695
GPIa (807C/T)	HET + HOM	132 (55.0)	13 (52.0)	0.89	0.36–2.18	0.774

CAG – coronary angiography, GP – glycoprotein, HET – variant allele in heterozygous form, HOM – variant allele in homozygous form, OR – odds ratio, PCI – percutaneous coronary intervention.

Table VI. Association between single nucleotide polymorphisms and gender

Identification of single nucleotide polymorphism	Allele type	Male *n* = 242 *n* (%)	Female *n* = 162 *n* (%)	Odds ratio	95% confidence interval	*P*-value
GPIa (807C/T)	HET + HOM	138 (57.3)	89 (55.3)	1.08	0.71–1.65	0.645
GPVI (13254T/C)	HET + HOM	55 (22.9)	27 (16.8)	1.47	0.85–2.53	0.140
P2Y12 (H1/H2 haplotype)	HET + HOM	57 (23.7)	41 (25.5)	0.91	0.56–1.48	0.678
P2Y12 (34C/T)	HET + HOM	135 (56.3)	90 (55.9)	1.01	0.66–1.55	0.945

GP – glycoprotein, HET – variant allele in heterozygous form, HOM – variant allele in homozygous form.

The highest prevalence of the polymorphic allele together in its heterozygous and homozygous form was found for GPIa 807C/T (56.7%) in our study. GPIa as a part of the GPIa/IIa complex is responsible for binding platelets to collagen. The frequency of a variant allele (in CT or TT form) is reported in the range of 50–70% across clinical trials [16]. The frequency of the 807T allele was slightly higher in the group of patients who developed bleeding (OR = 1.29, 95% CI: 0.75–2.24, $p = 0.334$) in our study. The presence of the T allele was associated with a higher risk of intracranial bleeding in the latest study of Zeng *et al.* [17]. In contrast, some other studies have demonstrated an association between the presence of the variant allele T at locus 807 of the gene for GPIa with higher expression of the GPIa/IIa receptor on the platelet surface, leading to a higher risk of ischaemic events, especially in young patients with myocardial infarction [18, 19]. However, the linkage was not confirmed in a large meta-analysis summarizing all available data up to the year 2007 [20]. The higher risk of ischaemic events in association with implantation of an intracoronary stent and C>T polymorphism was also not verified [21].

GPVI is another important receptor binding platelets to collagen. The gene for GPVI is located on the chromosome locus 19q13.4 of the human genome [22]. Previous studies described impaired collagen adhesion related to GPVI deficiency [23, 24]. We investigated the 13254 locus for the GPVI gene and its polymorphism T>C. The replacement of nucleotide bases in that area causes proline to serine substitution at position 219; therefore the binding function of GPVI may be affected. There are a few trials studying the 13254T/C polymorphism in relation to higher risk of ischaemic events, with inconsistent results [25–27]. We did not find any differences in C allele frequency between the bleeding and non-bleeding group (OR = 0.82, 95% CI: 0.40–1.64, $p = 0.551$). The homozygous form was rare (1.5%), with a similar rate in both groups (OR = 0.91, 95% CI: N/A, $p = 0.928$). The affinity of the external part of the GPVI receptor to collagen, where the proline to serine substitution is located, is probably not affected by this type of polymorphism enough to cause clinically significant haemostasis deficiency by itself.

The platelet receptor P2Y12, participating in platelet activation and aggregation, is not only one of the key receptors in relation to the primary haemostasis, but it is also the site of action of many antiplatelet drugs (ADP antagonists). Genetic defects of the P2Y12 receptor are associated with impaired coagulation and a tendency to bleeding [10]. Five SNPs which occur with the frequency ≥ 5% were identified in the P2Y12 gene. Three of them are located in an intron area (i-139C > T, i-744 T > C, i-801insA) and two of them in an exon domain (c.52G > T, c.34T >C). Fontana *et al.* first described strong linkage disequilibrium among four of them and identified the major H1 haplotype (occurring in 86%) and the minor H2 haplotype (occurring in 14%) [28]. In the analysis of Kvasnicka *et al.*, the frequency of H2 haplotype in its heterozygous form was 25.92%, while the homozygous form was identified in 2.63% of healthy volunteers [29], similarly as in our population (H1/H2 haplotype occurred in 22.4%, H2/H2 haplotype in 2%). The H2 haplotype in heterozygous and homozygous form together was not more likely identified in the bleeding group (OR = 0.69, 95% CI: 0.35–1.36, $p = 0.258$). When only the frequencies of the homozygous form were compared, in which the phenotypic penetration is assumed to be stronger, the frequency of the H2/H2 haplotype was higher in patients who developed periprocedural bleeding but the results did not reach the level of statistical significance (OR = 2.79, 95% CI: 0.51–13.77, $p = 0.161$). Compared to our study in which clinical findings were evaluated, the results of another study where only the laboratory data were assessed support our findings. Measured platelet activation and aggregation were significantly decreased in the H2 haplotype carriers, of which the lowest thrombin receptor-activating peptide (TRAP) induced platelet activation was found in H2/H2 haplotype individuals [30].

Another P2Y12 receptor polymorphism, 34C>T, is located in exon 2 and the linkage was not determinant for any type of haplotype. Variant allele T occurs in high frequency. The occurrence in our study population was 56.2% and in homozygous form the SNP occurred in 10.2%. These results correlate with the available data although the studies in this field are not numerous. One of them showed a higher incidence of neurological events in relation to the variant allele [31]. Another study, measuring laboratory platelet activity, determined the frequency of CT 41.8% and TT 5.7%. In that study, there was not found any influence of variant allele T presence and the maximal ADP-induced platelet aggregation [32]. The most recent trial of the clinical impact of P2Y12 (34C>T) demonstrated that minor allele carriage was associated with a significantly higher risk of bleeding after PCI in patients with ST elevation myocardial infarction (STEMI; OR = 2.71, 95% CI: 1.298–5.659, $p = 0.008$) [33]. We have not discovered higher risk of periprocedural bleeding in connection with the presence of the variant allele T in our study, where an unselected population of patients with ischaemic heart disease undergoing CAG or PCI was enrolled (OR = 0.71, 95% CI: 0.42–1.22, $p = 0.189$).

The limitation of most of the studies dealing with genetic variations in association with bleeding is that those studies are conducted on a small sample of patients, thereby creating a number of conflicting results. The number of patients in our study corresponds to several thousands of catheterized individuals, and thus it ranks among the largest studies on this theme. It would be most beneficial to undertake a large genome-wide association study, which have in the past significantly contributed to the understanding of genetic variations associated with the risk of cardiovascular diseases. Apart from cardiovascular diseases which are the leading cause

of morbidity and mortality in developed countries, the number of patients undergoing invasive procedures complicated with periprocedural bleeding is limited. Another factor contributing to the complexity of this issue is the intricacy of the phenotype penetration of SNPs, which further increases the demand on population size and statistical verifiability. Nevertheless, the direction is certainly appropriate, and it may, along with the stratification algorithms, contribute to more accurate identification of patients at higher risk of periprocedural bleeding in the future.

There are several limitations that should be addressed. The major limitation is the small sample size. The rate of bleeding complications, particularly the most serious, reported in notable recent major clinical trials, is low in general. As we evaluated both patients with acute coronary syndrome (ACS) and those with a stable form of ischaemic heart disease, and as in most participating centres the radial access is preferred, the incidence of bleeding is assumed to be even lower. If we were to anticipate the total number of patients who underwent CAG/PCI in our study according to the average bleeding complication rates from major clinical trials, it would equal several thousands of examined patients.

Secondly, patients with the most severe types of bleeding (intracranial or fatal bleeding) were not involved in the study because it was impossible to obtain informed content for genetic testing from those critically ill patients.

Conclusions

We demonstrated no increased risk of bleeding in the presence of polymorphisms of selected platelet receptors GPIa (807C>T, rs1126643), GPVI (13254T>C, rs1613662) and P2Y12 (34C>T, rs6785930 and H1/H2 haplotype, rs2046934) in patients undergoing CAG/PCI, although some findings suggest a particular linkage. Non-significantly higher frequency of P2Y12 H2 haplotype in its homozygous forms was found in patients with bleeding. Further studies are needed to verify this relationship.

Conflict of interest

The authors declare no conflict of interest.

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