Effect of common single-nucleotide polymorphisms in acetylsalicylic acid metabolic pathway genes on platelet reactivity in patients with diabetes

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Background: Platelet reactivity in patients on acetylsalicylic acid (ASA) therapy can be influenced by physiological or pathological conditions affecting ASA pharmacokinetics or pharmacodynamics. The mechanism of such variability in the therapeutic response to ASA, particularly in diabetic patients, is poorly understood. The rate of elimination of ASA and its metabolite, salicylic acid (SA), is likely a major factor determining drug efficacy. The objective of this study was to investigate the effect of genetic polymorphisms in the selected candidate genes within the ASA metabolic pathway on the platelet reactivity and concentration of ASA and thromboxane A₂ (TxA₂) metabolites in a population of patients with type 2 diabetes mellitus (T2DM).

Material/Methods: The study cohort consisted of 287 Caucasians with T2DM who had been taking ASA tablets at the dose of 75 mg per day for at least 3 months. Platelet reactivity analyses were performed using VerifyNow Aspirin and PFA-100 assays. The measured ASA metabolite included salicylic acid (ASA), and TxA₂ metabolites included serum TxB₂ and urinary 11-dih-TxB₂. Genotyping for the selected 18 single-nucleotide polymorphisms (SNPs) within 5 genes of the ASA metabolic pathway was performed using a Sequenom iPLEX platform.

Results: No statistically significant association was observed between the investigated SNPs genotypes, platelet reactivity, and measured metabolites in the investigated cohort of patients.

Conclusions: The results of our study failed to confirm that the selected variants in the genes within the ASA metabolic pathway might contribute to platelet reactivity in a diabetic population treated with ASA.

Key words: acetylsalicylic acid • cyclooxygenase-1 • diabetes mellitus • single-nucleotide polymorphisms • thromboxane • salicylic acid

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Background

Acetylsalicylic acid (ASA) inhibits platelet aggregation through irreversible acetylation of platelet cyclooxygenase (COX)-1 and subsequent inhibition of the metabolism of arachidonic acid (AA) into thromboxane A<sub>2</sub> (TxA<sub>2</sub>), a potent vasoconstrictor and aggregating agent [1]. ASA is an effective inhibitor of platelet TxA<sub>2</sub> production, nevertheless is often considered as a relatively weak platelet inhibitor. Moreover, platelet reactivity in patients on ASA therapy can be influenced by physiological or pathological conditions affecting ASA pharmacokinetics or pharmacodynamics. The mechanism of such variability in the therapeutic response to ASA, particularly in diabetic patients, is poorly understood [2–6]. The rate of elimination of ASA and its metabolite, salicylic acid (SA), is likely a major factor determining drug efficacy. It is possible that an increased hydrolysis of circulating ASA, which corresponds to faster elimination of the drug from the circulation by degradation into SA and acetate, may cause an altered response to ASA, and thus an increased platelet reactivity [6,7].

Oral bioavailability of ASA is approximately 50% because a substantial fraction of the administered dose is inactivated. ASA is rapidly deacetylated to SA in the liver, and to a lesser extent in the stomach, before entering the systemic circulation by a nonspecific human carboxylesterase 2 (HCE2) [8]. Other enzymes involved in ASA metabolism include 3 major enzymes: UDP-glucuronosyltransferase 1A6 (UGT1A6), xenobiotic/medium chain fatty acid: CoA ligase (ACSM2B), and cytochrome P450 2C9 (CYP2C9) [9]. Hydroxylation of SA, a minor metabolic pathway, is accomplished by cytochrome CYP2C9 to form gentistic acid, whereas UGT enzymes convert SA to glucuronides [salicyl acid phenolic glucuronide (SAPG) and salicyl acid acyl glucuronide (SAAG)], and salicyluric acid (SUA) phenolic glucuronide (SUAPG) after glycine conjugation [10]. Another ASA minor metabolite, salicyluric acid, is formed from salicylic acid, ATP, and coenzyme A in a reaction catalyzed by ACSM2B (Figure 1) [9]. Therefore, glucuronides represent a substantial proportion of ASA metabolites [10].

Several genetic variants that result in altered enzyme activity have been identified in the UGT1A family [11,12]. Polymorphisms within the genes coding for these enzymes may play a significant role in ASA pharmacokinetics and pharmacodynamics. The amino acid changes (T181A) and 184 (R184S) result in a 30–50% reduced enzyme activity compared with the wild-type allele [13]. The variant alleles for CYP2C9 (R144C and I359L) also produce enzymes characterized by only 5–30% of the activity of the wild-type enzyme [14].

The aim of this study was to determine whether changes in PFA-100 collagen/epinephrine closure time (CEPI-CT) and/or collagen/adenosine-5’-diphosphate (ADP) closure time (CADP-CT) and/or VerifyNow aspirin reaction units (ARU) are associated with SNPs in the candidate genes associated with ASA metabolism in a population of patients with T2DM. Our study represents a novel attempt to evaluate the platelet-related activity of the majority of known SNPs reported in ASA metabolism-associated genes in an exclusively diabetic population.

Material and Methods

Patient population and study design

The local ethics committee of the Medical University of Warsaw approved both the study protocol and the informed consent form. The study was conducted in accordance with the current version of the Declaration of Helsinki at the time when the study was designed, and informed written consent was obtained from all patients. The study subjects were recruited consecutively from patients with T2DM participating in a multi-center, prospective, randomized, and open-label AVOCADO [Aspirin Vs/Or Clopidogrel in Aspirin-resistant Diabetics inflammation Outcomes] study.
study, presenting to the outpatient clinic of the Central Teaching Hospital of the Medical University of Warsaw. The full characterization of the study population, including the inclusion and exclusion criteria, were published previously [15]. Briefly, 304 subjects with T2DM were recruited who at the time of enrollment had been taking ASA tablets at the dose of 75 mg per day for at least 3 months for primary or secondary prevention of myocardial infarction (MI). Clopidogrel or antiplatelet drugs other than ASA were not used in any of the investigated patients. All patients had been taking oral antidiabetic agents and/or insulin for at least 6 months; diet-controlled diabetic patients were not included. Compliance with ASA therapy was determined at study entry based upon the patient’s own statement and serum thromboxane B₂ (S-TxB₂) level measurement.

**Blood sample and assay procedures**

Blood samples were collected in the morning after an overnight fast and 2–3 hours after the last ASA dose. Blood was obtained from the antecubital vein, and the initial 2 ml of blood were discarded to avoid spontaneous platelet activation. Blood was drawn into tubes containing 3.2% sodium citrate for VerifyNow measurements and 3.8% sodium citrate for PFA-100 measurements. All blood samples were processed within 2 h of collection. Whole blood for S-TxB₂ was allowed to clot at 37°C for 1 h before separating serum by centrifugation. Serum was obtained from venous blood by centrifugation at 1000 g for 15 min at 4°C, and aliquots were stored at −80°C for further analysis.

**HPLC method for determination of ASA and its metabolites in plasma**

The concentration of ASA and its metabolites in plasma was measured by the method described by Krivosikova et al [16]. A high-performance liquid chromatograph system (Knauer, Germany) was equipped with a UV variable wavelength diode array detector and C18 reverse phase column 150×3.9 mm (Waters). The mobile phase consisted of water-85% phosphoric acid-butanol-tetrabutylammoniumhydroxide-methanol (134:1:1:63). The flow rate was 0.9 ml/min, the system was heated to 45°C, and the wavelength of detector was set at 237 nm. Salicylic acid, gentisic acid (GA) (Sigma), and salicyluric acid (SUA) (all from Roth) were used as calibration standards. The chromatograms analysis was performed with ClarityChrom Software (Knauer, Germany) [16].

The concentration of functional epitope of the vWF molecule (vWF: Ag) was measured in citrate plasma samples using an enzyme immunoassay kit according to the manufacturer’s instructions (vWF Activity Kit, American Diagnostica Inc., USA). S-TxB₂ concentrations were also measured with an enzyme immunoassay kit according to the manufacturer’s instructions (EIA kits, Cayman Chemicals, Ann Arbor, MI, USA). Each lot of TxB₂, EIA kits was tested for the impact of interferences. The correlation of results in 3 dilutions of 5 random samples was assessed, as was proposed in the kit protocol. The decision to use the assay without purification was taken after analysis of results because differences of results did not exceed 20%. Samples with results outside the standard curve were re-assayed with appropriate dilutions. An optimal compliance was confirmed by S-TxB₂ levels below 7.2 ng/mL in all patients as described previously in a diabetic population by Mortensen et al [17].

A first morning urine specimen was collected and brought in by the patient within 2 h of collection. The samples were collected into tubes containing indomethacin and then were stored at −80°C for further analysis. Urinary 11-dh-TxB₂ concentrations were measured using an enzyme immunoassay kit according to the manufacturer’s instructions (EIA kits, Cayman Chemicals, Ann Arbor, MI, USA) after extraction and purification on SPE (C18) columns (Waters Associates, Milford, MA, USA), and data were normalized for urinary creatinine concentration.

**Analysis of platelet functions**

Platelet reactivity was measured with VerifyNow Aspirin Assay (Accumetrics, San Diego, CA, USA) and PFA-100 assay (Dade Behring International, Inc., Newark, DE, USA). These assays were performed as described in detail previously [15]. Based on our own and other previous reports, we used 3 different cut-off values for high platelet reactivity in the CEPI-CT assay, but we did not specify such a point for collagen/adenosine diphosphate (CADP)-CT. In the first approach, adequate platelet inhibition with ASA was defined as CEPI-CT ≥165 s, and in the second as CEPI-CT ≥193 s (the manufacturer’s suggested lower limit of the normal range for aspirin-free healthy controls) [18]. The maximum CT given for PFA-100 is 300 s and is equivalent to non-occlusion [19]. Thus, patients with CEPI-CT values ≥300 s were defined as an alternative population with adequate platelet inhibition [20]. According to the manufacturer, ARU ≥550 indicates no effect of ASA on platelet aggregation, whereas ARU <550 indicates platelet dysfunction due to inhibition of the COX1-dependent pathway [21].

**DNA extraction, quality control, and quantification**

DNA was obtained from frozen whole blood samples stored until the time of analysis using the membrane ultrafiltration method using a Fuji MiniGene 80 extractor (FujiFilm Life Sciences, distributed by Autogene, Holliston, MA, USA). DNA concentrations were determined spectrophotometrically using a PicoGreen dsDNA Quantitation Reagent Kit (Molecular Probes Inc., Eugene, OR, USA).
Individual SNP genotyping

Genotyping was performed at Children’s Hospital Boston using a custom Sequenom iPLEX assay in conjunction with the Mass ARRAY platform (Sequenom Inc., La Jolla, CA, USA). One panel of SNP markers was designed using Sequenom Assay Design 3.2 software.

Statistical analysis of results

Power analysis

We planned a prospective, observational study of diabetic patients treated with ASA. Hardy-Weinberg equilibrium was evaluated using the 2-tailed chi-square test. The primary analysis used the 2-tailed chi-square test followed by univariate logistic regression with genotypes for each SNP as dependent variable, and VerifyNow ARU >550 phenotype (high on ASA platelet reactivity) as binary independent variable. The logistic regression procedure enabled us to estimate the log of the odds ratio (OR), a measure of the increase in odds of experiencing VerifyNow ARU >550 for subjects carrying the variant compared to the wild-type subjects. We obtained a 95% confidence interval around this estimate and the p value for the OR. The p value was compared with the pre-defined cutoff for statistical significance (alpha =0.05/number of investigated SNPs and outcome phenotypes =0.0028 because of nominal alpha level 0.05 corrected by Bonferroni method for 13 simultaneously analyzed polymorphisms and 5 outcome phenotypes). Given the expected population incidence of VerifyNow ARU >550 in the investigated diabetic population of approximately 20%, average allele frequency of minor allele 0.2, alpha level =0.0028, and the power of 0.8, the study needed at least 280 subjects to detect clinically significant OR=3 for experiencing VerifyNow ARU >550 in carriers of the minor allele.

Statistical calculations

The statistical analyses were performed using IBM-SPSS ver. 19 and Stata (Stata Corporation, College Station, TX) software. Deviations from Hardy-Weinberg equilibrium (HWE) were calculated using the chi-square test. The recorded clinical data, when normally distributed in the analyzed group of patients, were presented as mean and SD, and non-normally distributed data are presented as medians and interquartile range (IQR). We compared the distribution of predefined cut-off values for both assays (i.e., CEPI-CT for PFA-100 [<165 s, <193 s and <300 s] and ARU for VerifyNow [≤550]) using exact chi-square statistics and distribution of medians (i.e., CEPI-CT and CADP-CT for PFA-100 and ARU for VerifyNow) among all genotypes of successfully genotyped SNPs for each of 3 genotypes (i.e., homozygotes for minor allele, heterozygotes and homozygotes

| Table 1. Demographic and clinical characteristics of the study patients (N=284). |
|---------------------------------------------|
| Demographics |
| Age (years) | 67.6±8.7 |
| Female | 135 (53.5%) |
| BMI | 31.1±12.0 |
| SBP (mmHg) | 142.3±18.9 |
| DBP (mmHg) | 80.5±11.3 |
| Dyslipidemia | 234 (82.4%) |
| CAD | 162 (57.0%) |
| Prior MI | 87 (30.6%) |
| Prior stroke | 23 (8.1%) |
| Heart failure | 107 (37.7%) |
| History of smoking | 160 (56.3%) |
| Current smoking | 78 (27.0%) |

Concurrent medications

- Oral hypoglycemics | 243 (85.6%) |
- Insulin | 93 (32.7%) |
- Beta-blockers | 205 (72.2%) |
- ACE inhibitors | 185 (65.1%) |
- Statins | 206 (72.6%) |

Biochemical and hematological parameters

- HGB | 13.8±1.3 |
- HCT | 41.3±4.5 |
- WBC | 7.1±2.2 |
- PLT | 227.8±58.3 |
- MPV | 9.9±1.2 |
- eGFR | 70.8±20.9 |
- HbA1c | 7.0±1.3 |
- hsCRP | 4.1±5.6 |
- CEPI-CT | 266.5 [IQR 129] |
- CADP-CT | 97.0 [IQR 49] |
- VerifyNow (ARU) | 456.5 [IQR 97.5] |
- S-TxB2 | 102.585 [IQR 40.45] |
- SA | 0.091 [IQR 0.362] |

Data are expressed as mean (±SD) unless otherwise indicated. Abbreviations: BMI – body mass index (kg/m²); SBP – systolic blood pressure; DBP – diastolic blood pressure; CAD – coronary artery disease; MI – myocardial infarction; ACE – angiotensin-converting enzyme; HGB – hemoglobin (g/dL); HCT – hematocrit (%); WBC – white blood cells (10³/mm³); PLT – platelet count (10⁹/mm³); MPV – mean platelet volume (fl); eGFR – estimated glomerular filtration rate (mL/min/1.73m²); HbA1c – glycated hemoglobin (%); hsCRP – high sensitivity C-reactive protein (mg/L); CEPI-CT – collagen/epinephrine closure time (sec); CADP-CT – collagen/adenosine diphosphate closure time (sec); ARU – aspirin reaction units; IQR – interquartile range; S-TxB2 – serum thromboxane B2 (ng/mL); 11-dTxB2 – 11-Dehydrothromboxane B2 (ng/mg Cr); SA – salicylic acid (ng/µL).
for major allele) using the Kruskal-Wallis test. The SNPs were considered statistically significant when \( p < 0.05/30 \) (i.e., \( p \) corrected for multiple comparisons).

The SNPs with nominal statistically significant (i.e., \( p < 0.05 \) before applying correction for multiple comparisons) differences in the measurements for platelet reactivity (PFA-100 CEPITC and VerifyNow ARU) between pre-defined cutoff value for each of 3 genotypes (i.e., homozygotes for minor allele, heterozygotes and homozygotes for major allele) were subjected to further testing based on the dominant, recessive, or additive genetic model by use of the Mann-Whitney test.

**Results**

From the initially enrolled 304 patients, complete clinical data and blood samples finally became available for 298 patients. Subsequently, 8 patients were eliminated from the analysis based on the suspected ASA non-compliance (S-TxB\(_2\) concentrations \( >7.2 \) ng/ml). A further 3 patients were eliminated because of the lack of corresponding biochemical and genotype data. Demographic characteristics, clinical data, and results of platelet reactivity measurements, serum concentrations of S-TxB\(_2\), and SA, urine concentrations of 11-dh-TxB\(_2\) data for the remaining 287 patients are summarized in Table 1.

Genotyping was attempted for 18 initially selected SNPs (Figure 1). One SNP genotyped poorly (cut-off <85%) (rs1634312, ACSM2A gene) and 4 SNPs were not included into the final analysis because we found only homozygotes in our population (rs28371685, rs28371686, rs9332131, CYP2C9 gene; rs3893757, CES2 gene). The remaining 17 SNPs genotyped well (>86% success rate) and were in Hardy-Weinberg equilibrium. The summary results of the allele and genotype frequencies for all genotyped SNPs included into the final analysis are summarized in Table 2.

For each of the successfully genotyped SNPs, we initially compared the corresponding platelet reactivity measurements (i.e., CEPITC and CDP-CT for PFA-100 and ARU for VerifyNow), S-TxB\(_2\), and serum SA concentrations and urine concentrations of 11-dh-TxB\(_2\) between carriers and non-carriers of the

| Gene name (SNP rs#) | Allele frequency | Genotypes frequency | HWE P value |
|---------------------|------------------|---------------------|-------------|
| ACSM2 (rs28750179)  | A (.128); G (.872)| AA (.031); AG (.199); GG (.770) | .26         |
| ACSM3 (rs5716)      | C (.078); G (.922)| CC (.011); CG (.134); GG (.855) | .7          |
| ACSM5 (rs99228053)  | A (.017); G (.983)| AG (.034); GG (.966) | .96         |
| ACSM5 (rs71922110)  | A (.016); G (.984)| AG (.033); GG (.967) | .96         |
| ACSM5 (rs5713)      | C (.007); T (.993)| CT (.014); TT (.986) | .99         |
| UGT1A6 (rs17863783) | T (.012); G (.988)| TG (.024); GG (.976) | .97         |
| UGT1A6 (rs6759892)  | G (.469); T (.531)| GG (.234); GT (.469); TT (.297) | .58         |
| UGT1A6 (rs1105800)  | G (.412); A (.588)| GG (.190); AG (.444); AA (.366) | .31         |
| UGT1A6 (rs2070959)  | G (.400); A (.600)| GG (.176); AG (.448); AA (.376) | .4          |
| CYP2C9 (rs28371685) | C (.100)         | CC (.100)            | .55         |
| CYP2C9 (rs9332108)  | C (.055); T (.945)| C (.003); CT (.104); TT (.893) | 1           |
| CYP2C9 (rs1057911)  | T (.055); A (.945)| TT (.003); AT (.104); AA (.893) | 1           |
| CYP2C9 (rs28371686) | C (.100)         | CC (.100)            | .55         |
| CYP2C9 (rs9332131)  | A (.100)         | AA (.100)            | .99         |
| CES2 (rs3893757)    | C (.100)         | CC (.100)            | .55         |
| CES2 (rs8061994)    | A (.108); G (.892)| AA (.013); AG (.188); GG (.799) | .97         |
| CES2 (rs58404026)   | G (.031); C (.969)| CG (.062); CC (.938) | .87         |

HWE – Hardy-Weinberg equilibrium.
Table 3. Genotypes distribution (dominant model) for predefined cut-off values for VerifyNow (ARU>550) using exact chi-square statistics.

| Analyzed SNPs in ASA metabolism pathway | VerifyNow ARU | P* (chi-square test) |
|----------------------------------------|--------------|---------------------|
| rs28750179 G>A                         |              |                     |
| Homozygotes for major allele (N)       | 38           | 0.493               |
| Homozygotes and                        |              |                     |
| heterozygotes and                      | 9            |                     |
| homozygotes for minor variant allele   | 138          |                     |
| (N)                                    |              |                     |
| P* (chi-square test)                   | 0.493        |                     |
| rs5716 G>C                             |              |                     |
| Homozygotes for major allele (N)       | 6            | 0.692               |
| Homozygotes and                        |              |                     |
| heterozygotes and                      | 41           |                     |
| homozygotes for minor variant allele   | 204          |                     |
| (N)                                    |              |                     |
| P* (chi-square test)                   | 0.579        |                     |
| rs99228053 G>A                         |              |                     |
| Homozygotes for major allele (N)       | 46           | 0.579               |
| Homozygotes and                        |              |                     |
| heterozygotes and                      | 0            |                     |
| homozygotes for minor variant allele   | 231          |                     |
| (N)                                    |              |                     |
| P* (chi-square test)                   | 0.579        |                     |
| rs7192210 G>A                          |              |                     |
| Homozygotes for major allele (N)       | 42           | 0.192               |
| Homozygotes and                        |              |                     |
| heterozygotes and                      | 1            |                     |
| homozygotes for minor variant allele   | 221          |                     |
| (N)                                    |              |                     |
| P* (chi-square test)                   | 0.192        |                     |
| rs5713 T>C                             |              |                     |
| Homozygotes for major allele (N)       | 46           | 0.639               |
| Homozygotes and                        |              |                     |
| heterozygotes and                      | 1            |                     |
| homozygotes for minor variant allele   | 237          |                     |
| (N)                                    |              |                     |
| P* (chi-square test)                   | 0.639        |                     |
| rs17863783 G>T                         |              |                     |
| Homozygotes for major allele (N)       | 46           | 0.880               |
| Homozygotes and                        |              |                     |
| heterozygotes and                      | 1            |                     |
| homozygotes for minor variant allele   | 234          |                     |
| (N)                                    |              |                     |
| P* (chi-square test)                   | 0.880        |                     |
| rs6759892 T>G                           |              |                     |
| Homozygotes for major allele (N)       | 13           | 0.884               |

N – number of carriers for each genotype; ARU – Aspirin Reaction Unit; * P using chi-square test for differences between 2 analyzed genotypes for each SNP.
Table 4. Genotypes distribution (dominant model) for predefined cut-off values for S-TxB₂, 11-dh-TxB₂ and serum SA concentrations using exact chi-square statistics.

| Analyzed SNPs in ASA metabolism pathway | S-TxB₂ (ng/ml) | 11-dh-TxB₂ (ng/mg Cr) | SA (ng/μl) |
|----------------------------------------|----------------|------------------------|------------|
|                                        | <0.153         | ≥0.153                 | <102.585   | ≥102.585 | <0.091 | ≥0.091 |
| rs28750179 G>A                         |                |                        |            |
| Homozygotes [major allele] (N)         | 108            | 112                    | 95         | 100     | 114    | 110    |
| Hetero-, homozygotes [minor (variant) allele] (N) | 33             | 30                     | 33         | 27      | 30     | 36     |
| P* (chi-square test)                  | 0.645          | 0.395                  | 0.437      |
| rs5716 G>C                            |                |                        |            |
| Homozygotes [major allele] (N)         | 16             | 25                     | 22         | 14      | 20     | 22     |
| Hetero-, homozygotes [minor (variant) allele] (N) | 125            | 117                    | 106        | 113     | 124    | 124    |
| P* (chi-square test)                  | 0.135          | 0.758                  | 0.775      |
| rs99228053 G>A                        |                |                        |            |
| Homozygotes [major allele] (N)         | 138            | 135                    | 123        | 122     | 141    | 139    |
| Hetero-, homozygotes [minor (variant) allele] (N) | 3             | 7                      | 5          | 5       | 3      | 7      |
| P* (chi-square test)                  | 0.202          | 0.990                  | 0.206      |
| rs7192210 G>A                         |                |                        |            |
| Homozygotes [major allele] (N)         | 130            | 129                    | 117        | 115     | 135    | 131    |
| Hetero-, homozygotes [minor (variant) allele] (N) | 3            | 6                      | 5          | 4       | 3      | 6      |
| P* (chi-square test)                  | 0.320          | 0.763                  | 0.304      |
| rs5713 T>C                            |                |                        |            |
| Homozygotes [major allele] (N)         | 140            | 139                    | 126        | 125     | 141    | 145    |
| Hetero-, homozygotes [minor (variant) allele] (N) | 1            | 3                      | 2          | 2       | 1      | 1      |
| P* (chi-square test)                  | 0.317          | 0.994                  | 0.307      |
| rs17863783 G>T                         |                |                        |            |
| Homozygotes [major allele] (N)         | 137            | 139                    | 125        | 124     | 141    | 142    |
| Hetero-, homozygotes [minor (variant) allele] (N) | 4            | 3                      | 3          | 3       | 3      | 4      |
| P* (chi-square test)                  | 0.695          | 0.992                  | 0.716      |
| rs6759892 T>G                          |                |                        |            |
| Homozygotes [major allele] (N)         | 37             | 46                     | 36         | 38      | 46     | 42     |
| Hetero-, homozygotes [minor (variant) allele] (N) | 104           | 96                     | 92         | 89      | 100    | 104    |
| P* (chi-square test)                  | 0.256          | 0.752                  | 0.739      |
| rs1105880 A>G                          |                |                        |            |
| Homozygotes [major allele] (N)         | 48             | 54                     | 43         | 48      | 50     | 56     |
| Hetero-, homozygotes [minor (variant) allele] (N) | 93            | 88                     | 85         | 79      | 88     | 96     |
| P* (chi-square test)                  | 0.485          | 0.484                  | 0.412      |
investigated SNP variants after correction for multiple comparisons. We did not find any nominal statistically significant results for groups of patients based on predefined cut-off values for platelet reactivity assays (CEPI-CT, CADP-CT, and ARU) (Tables 3 and 4, and Supplemental Table 3).

**Discussion**

The present study examined the association between variants in multiple genes related to ASA metabolism in a diabetic population with high ASA platelet reactivity phenotypes during chronic ASA therapy in a homogeneous diabetic population from central Poland. In particular, we characterized a group of SNPs for each gene, selected on the basis of earlier reports [9–11,13,28–30]. For the assessment of platelet reactivity, we used 3 different “point-of-care” assays that are commonly used in the evaluation of platelet reactivity.

We successfully genotyped 17 informative, common SNPs within 5 genes related to ASA metabolism: 5 SNPs in ACSM, 4 SNPs in UGT1A6, 5 SNPs in CYP2C9, and 3 SNPs in CES2. We found no association between SNPs in candidate genes and measured platelet reactivity, metabolites of AA (i.e., S-TxB$_2$, 11-dh-TxB$_2$), and ASA metabolite (i.e., SA) in a population of patients with T2DM. These findings suggest these genetic variations have no major effect of on inter-individual differences in the platelet reactivity related to ASA metabolism in the T2DM population.

We successfully genotyped 4 SNP within the UGT1A6 gene associated with the amino acid substitution in position 7 (rs6759892), 105 (rs1105880), 181 (rs2070959), and 209 (rs28371685). However, we failed to genotype another important SNP – rs1105879 – that causes amino acid substitution in position 184.

**Table 4 continued.** Genotypes distribution (dominant model) for predefined cut-off values for S-TxB$_2$, 11-dh-TxB$_2$, and serum SA concentrations using exact chi-square statistics.

| Analyzed SNPs in ASA metabolism pathway | S-TxB$_2$ (ng/ml) | 11-dh-TxB$_2$ (ng/mg Cr) | SA (ng/μl) |
|-----------------------------------------|------------------|--------------------------|-----------|
|                                         | <0.153 | ≥0.153 | <102.585 | ≥102.585 | <0.091 | ≥0.091 |
| rs2070959 A>G                           |        |        |          |          |        |        |
| Homozygotes [major allele] (N)          | 72     | 56     | 74       | 49       | 58     | 26     |
| Hetero-, homozygotes [minor (variant) allele] (N) | 92     | 86     | 83       | 78       | 86     | 95     |
| P* (chi-square test)                    | 0.415  | 0.571  | 0.347    |          |        |        |
| rs9332108 T>C                           |        |        |          |          |        |        |
| Homozygotes [major allele] (N)          | 127    | 126    | 113      | 117      | 124    | 134    |
| Hetero-, homozygotes [minor (variant) allele] (N) | 14     | 15     | 14       | 10       | 20     | 11     |
| P* (chi-square test)                    | 0.845  | 0.391  | 0.083    |          |        |        |
| rs1057911 A>T                           |        |        |          |          |        |        |
| Homozygotes [major allele] (N)          | 127    | 127    | 114      | 117      | 124    | 135    |
| Hetero-, homozygotes [minor (variant) allele] (N) | 14     | 15     | 14       | 10       | 20     | 11     |
| P* (chi-square test)                    | 0.860  | 0.080  | 0.080    |          |        |        |
| rs8061994 G>A                           |        |        |          |          |        |        |
| Homozygotes [major allele] (N)          | 114    | 111    | 106      | 93       | 111    | 119    |
| Hetero-, homozygotes [minor (variant) allele] (N) | 29     | 29     | 32       | 27       | 34     | 27     |
| P* (chi-square test)                    | 0.743  | 0.103  | 0.347    |          |        |        |
| rs58407626 C>G                          |        |        |          |          |        |        |
| Homozygotes [major allele] (N)          | 129    | 136    | 119      | 120      | 137    | 135    |
| Hetero-, homozygotes [minor (variant) allele] (N) | 13     | 5      | 9        | 7        | 7      | 11     |
| P* (chi-square test)                    | 0.053  | 0.617  | 0.346    |          |        |        |

N – number of carriers for each genotype; S-TxB$_2$ – serum thromboxane B$_2$ (ng/mL); 11-dh-TxB$_2$ – 11-Dehydro thromboxane B$_2$ (ng/mg Cr); SA – salicylic acid (ng/μl); * P using chi-square test for differences between 2 analyzed genotypes for each SNP.
Supplemental Table 1. The effects of different genotypes (dominant model) of analyzed SNPs in ASA metabolism pathway on plasma levels of S-TxB₂ and SA, and urine excretion of 11-dh-TxB₂ in diabetic patients on ASA therapy.

| Analyzed SNPs in ASA metabolism pathway | S-TxB₂ (ng/ml) | 11-dh-TxB₂ (ng/mg Cr) | SA (ng/μl) | P (MW test) |
|----------------------------------------|----------------|------------------------|------------|-------------|
| rs28750179 G>A                          |                |                        |            |             |
| Homozygotes for major allele (N=194)    | 0.191 (0.6)    | 41.100 (41.62)         | 0.088 (0.3) | 0.761       |
| Heterozygotes and homozygotes for minor (variant) allele (N=59) | 0.141 (1.0) | 33.200 (39.87) | 0.157 (1.0) | 0.729       |
| P (MW test)                             |                |                        |            |             |
| rs5716 G>C                              |                |                        |            |             |
| Homozygotes for major allele (N=36)     | 0.274 (0.8)    | 31.345 (40.01)         | 0.106 (0.6) | 0.963       |
| Heterozygotes and homozygotes for minor (variant) allele (N=217) | 0.159 (0.6) | 41.500 (41.46) | 0.092 (0.3) | 0.795       |
| P (MW test)                             | 0.093          | 0.115                  | 0.963      |             |
| rs99228053 G>A                          |                |                        |            |             |
| Homozygotes for major allele (N=243)    | 0.165 (0.6)    | 40.430 (40.61)         | 0.91 (0.4) | 0.115       |
| Heterozygotes and homozygotes for minor (variant) allele (N=10) | 0.267 (0.5) | 39.890 (52.51) | 0.253 (0.6) | 0.568       |
| P (MW test)                             | 0.568          | 0.795                  | 0.484      |             |
| rs7192210 G>A                           |                |                        |            |             |
| Homozygotes for major allele (N=230)    | 0.176 (0.6)    | 40.365 (40.31)         | 0.09 (0.4) |             |
| Heterozygotes and homozygotes for minor (variant) allele (N=9) | 0.239 (0.6) | 36.980 (57.03) | 0.387 (0.7) |             |
| P (MW test)                             | 0.670          | 0.815                  | 0.516      |             |
| rs5713 T>C                              |                |                        |            |             |
| Homozygotes for major allele (N=249)    | 0.176 (0.6)    | 40.430 (41.38)         | 0.096 (0.4) |             |
| Heterozygotes and homozygotes for minor (variant) allele (N=4) | 1.811 (2.2) | 42.300 (28.11) | 0.049 (0.1) |             |
| P (MW test)                             | 0.143          | 0.672                  | 0.217      |             |
| rs17863783 G>T                          |                |                        |            |             |
| Homozygotes for major allele (N=247)    | 0.176 (0.6)    | 40.430 (40.03)         | 0.092 (0.4) |             |
| Heterozygotes and homozygotes for minor (variant) allele (N=6) | 0.291 (1.1) | 41.145 (79.27) | 0.084 (0.7) |             |
| P (MW test)                             | 0.811          | 0.836                  | 0.958      |             |
| rs6759892 T>G                           |                |                        |            |             |
| Homozygotes for major allele (N=74)     | 0.203 (0.6)    | 40.490 (42.39)         | 0.088 (0.4) |             |
| Heterozygotes and homozygotes for minor (variant) allele (N=179) | 0.153 (0.6) | 40.200 (45.38) | 0.096 (0.3) |             |
| P (MW test)                             | 0.200          | 0.648                  | 0.467      |             |
| rs1105880 A>G                           |                |                        |            |             |
| Homozygotes for major allele (N=91)     | 0.200 (0.7)    | 40.700 (43.19)         | 0.082 (0.5) |             |
| Heterozygotes and homozygotes for minor (variant) allele (N=162) | 0.153 (0.5) | 39.055 (40.31) | 0.099 (0.3) |             |
| P (MW test)                             | 0.377          | 0.691                  | 0.514      |             |
Supplemental Table 1 continued. The effects of different genotypes (dominant model) of analyzed SNPs in ASA metabolism pathway on plasma levels of S-TxB₂ and SA, and urine excretion of 11-dh-TxB₂ in diabetic patients on ASA therapy.

| Analyzed SNPs in ASA metabolism pathway | S-TxB₂ (ng/ml) | 11-dh-TxB₂ (ng/mg Cr) | SA (ng/μl) |
|-----------------------------------------|----------------|------------------------|------------|
| rs2070959 A>G                           |                |                        |            |
| Homozygotes for major allele (N=94)     | 0.203 (0.7)    | 40.605 (46.17)         | 0.081 (0.4) |
| Heterozygotes and homozygotes for minor (variant) allele (N=159) | 0.153 (0.5) | 39.120 (39.87) | 0.101 (0.3) |
| P (MW test)                             | 0.274          | 0.876                  | 0.337      |
| rs9332108 T>C                           |                |                        |            |
| Homozygotes for major allele (N=228)    | 0.162 (0.6)    | 40.605 (43.18)         | 0.099 (0.4) |
| Heterozygotes and homozygotes for minor (variant) allele (N=24) | 0.322 (0.6) | 37.545 (27.71) | 0.066 (0.1) |
| P (MW test)                             | 0.861          | 0.368                  | 0.113      |
| rs1057911 A>T                           |                |                        |            |
| Homozygotes for major allele (N=229)    | 0.165 (0.6)    | 40.510 (43.03)         | 0.097 (0.4) |
| Heterozygotes and homozygotes for minor (variant) allele (N=24) | 0.322 (0.6) | 37.545 (27.71) | 0.123 (0.1) |
| P (MW test)                             | 0.841          | 0.376                  | 0.112      |
| rs8061994 G>A                           |                |                        |            |
| Homozygotes for major allele (N=197)    | 0.159 (0.6)    | 38.580 (38.44)         | 0.106 (0.5) |
| Heterozygotes and homozygotes for minor (variant) allele (N=54) | 0.210 (0.9) | 47.485 (39.26) | 0.073 (0.1) |
| P (MW test)                             | 0.569          | 0.155                  | 0.248      |
| rs58407626 C>G                          |                |                        |            |
| Homozygotes for major allele (N=237)    | 0.183 (0.6)    | 40.470 (39.58)         | 0.090 (0.4) |
| Heterozygotes and homozygotes for minor (variant) allele (N=16) | 0.086 (1.1) | 38.195 (71.68) | 0.216 (0.5) |
| P (MW test)                             | 0.228          | 0.947                  | 0.355      |

Data are shown as median and interquartile range (IQR); * P using Mann-Whitney (MW) test for differences between 2 analyzed genotypes for each SNP; ASA – acetylsalicylic acid; N – number of carriers for each genotype, MAF = minor allele frequency for each analyzed SNP in investigated cohort.

Variability in glucuronidation activity among ASA users is probably a result of specific expression levels and/or functional polymorphisms present in the UGTs catalyzing the conjugation of salicylic acid. To date, polymorphisms in many of the UGT enzymes have been identified [29]. UGT1A6*2 contains 2 missense mutations in exon 1 that result in T181A and R184S amino-acid substitution. These substitutions may be critical for ASA efficacy and are associated with altered enzyme function [12,22]. Allele frequencies of 17–33% for this variant in predominantly Caucasian populations have been reported [11,13]. An association with decreased risk of colorectal adenoma has been described among patients on ASA therapy with a UGT1A6 variant allele [10,14,23]. Consistent with these findings, Ciotti et al (1997) demonstrated that UGT1A6 was able to catalyze the glucuronidation of salicylic acid, with expressed UGT1A6*2 demonstrating a 2-fold lower salicylic acid glucuronidation compared with UGT1A6*1 [11].

The effects of inter-individual differences in UGT1A6 and CYP2C9 genotypes on ASA metabolism have been described in colon adenoma [15]. Moreover, impairment in CYP2C9 metabolism contributes to risk of developing gastrointestinal complications with aspirin use [24]. Although many variant alleles within the CYP2C9 gene exist, most of these are uncommon or do not cause a relevant effect on enzyme activity [25]. It should be also noted that great inter-ethnic and intra-ethnic variability were observed in the frequencies of SNPs for common CYP2C9. We genotyped 3 SNPs that cause the amino acid substitution in position 360 (rs28371686), 273 (rs9332131), and 335 (rs28371685) that are associated with decreased drug...
Supplemental Table 2. The effects of different genotypes (dominant model) of analyzed SNPs in ASA metabolism pathway on platelet reactivity measured with VerifyNow and PFA-100 (CEPI-CT and CADP-CT) in diabetic patients on ASA therapy.

| Analyzed SNPs in ASA metabolism pathway | VerifyNow (ARU) | CEPI-CT (sec.) | CADP-CT (sec.) |
|----------------------------------------|----------------|----------------|----------------|
| rs28750179 G>A                         |                |                |                |
| Homozygotes for major allele (N=218)   | 456 (98)       | 275 (129)      | 96 (47)        |
| Heterozygotes and homozygotes for minor (variant) allele (N=64) | 451.5 (101) | 255 (134) | 100 (75) |
| P (MW test)                            | 0.706          | 0.255          | 0.426          |
| rs5716 G>C                             |                |                |                |
| Homozygotes for major allele (N=40)    | 479 (119)      | 300 (136)      | 95.5 (123)     |
| Heterozygotes and homozygotes for minor (variant) allele (N=242) | 455 (95) | 262 (129) | 97.5 (45) |
| P (MW test)                            | 0.471          | 0.273          | 0.609          |
| rs99228053 G>A                         |                |                |                |
| Homozygotes for major allele (N=273)   | 455 (98)       | 265 (129)      | 99 (49)        |
| Heterozygotes and homozygotes for minor (variant) allele (N=9) | 486 (82) | 193 (139) | 78 (35) |
| P (MW test)                            | 0.172          | 0.593          | 0.370          |
| rs7192210 G>A                          |                |                |                |
| Homozygotes for major allele (N=259)   | 450 (98)       | 268 (130)      | 97 (49)        |
| Heterozygotes and homozygotes for minor (variant) allele (N=8) | 479 (75) | 239.5 (126) | 84 (41) |
| P (MW test)                            | 0.295          | 0.848          | 0.562          |
| rs5713 T>C                             |                |                |                |
| Homozygotes for major allele (N=275)   | 456 (99)       | 263 (130)      | 97 (49)        |
| Heterozygotes and homozygotes for minor (variant) allele (N=4) | 485.5 (59) | 287 (129) | 91.5 (171) |
| P (MW test)                            | 0.195          | 0.659          | 0.919          |
| rs17863783 G>T                         |                |                |                |
| Homozygotes for major allele (N=275)   | 456 (99)       | 263 (130)      | 97 (49)        |
| Heterozygotes and homozygotes for minor (variant) allele (N=7) | 502 (75) | 300 (87) | 100 (60) |
| P (MW test)                            | 0.525          | 0.229          | 0.859          |
| rs6759892 T>G                          |                |                |                |
| Homozygotes for major allele (N=82)    | 452.5 (94)     | 276.5 (128)    | 96.5 (51)      |
| Heterozygotes and homozygotes for minor (variant) allele (N=200) | 456.5 (102) | 262 (130) | 97.5 (49) |
| P (MW test)                            | 0.973          | 0.784          | 0.890          |
| rs1105880 A>G                          |                |                |                |
| Homozygotes for major allele (N=100)   | 458.5 (100)    | 270.5 (141)    | 96 (52)        |
| Heterozygotes and homozygotes for minor (variant) allele (N=182) | 454 (99) | 264 (128) | 98.5 (47) |
| P (MW test)                            | 0.298          | 0.900          | 0.945          |
metabolism. However, in our cohort, only homozygotes for major allele were found for all of them.

When compared to other genes described in this study, the observed variability in the ACSM2B gene is extremely low. Other ACSM2 genes have been identified and, because the enzymes encoded by these genes may participate in the glycine conjugation of salicylic acid, a relevant effect of SNPs affecting these genes cannot be ruled out [9]. Because no common and functionally significant non-synonymous SNPs have been described to date, it is not surprising that we did not observed any association with studied phenotypes.

ASA is predominately hydrolyzed by HCE2. CES2 appears to have very little genetic variation, with the majority of reported SNPs occurring in intronic regions [26]. In Japanese subjects, a total of 21 SNPs within the CES2 gene were identified, but some of them are infrequent in the studied population [27]. Marsh et al identified 10 SNPs in a European population, but only 3 of them have minor allele frequency (MAF) >0.025 [26]. In our study, we found 2 SNPs within CES2 with MAF >0.025 (rs8061994 and rs58407626), but we found all studied genotypes (homozygotes for minor allele, heterozygotes and homozygotes for major allele) only for rs8061994.

The lack of observed association between investigated SNPs in the ASA metabolism-pathway and platelet function phenotypes, AA metabolites, and ASA metabolites in our study could be explained by the limited number of investigated variants, which are only a small fraction of all previously reported variants with selected genes associated with ASA metabolism. It is possible that the selected variants could, in fact, be involved in the

Supplemental Table 2. The effects of different genotypes (dominant model) of analyzed SNPs in ASA metabolism pathway on platelet reactivity measured with VerifyNow and PFA-100 (CEPI-CT and CADP-CT) in diabetic patients on ASA therapy.

| Analyzed SNPs in ASA metabolism pathway | VerifyNow (ARU) | CEPI-CT (sec.) | CADP-CT (sec.) |
|----------------------------------------|----------------|----------------|----------------|
| rs2070959 A>G                          |                |                |                |
| Homozygotes for major allele (N=103)   | 459 (101)      | 273 (136)      | 96 (52)        |
| Heterozygotes and homozygotes for minor (variant) allele (N=179) | 453 (99) | 263 (129) | 98 (47) |
| P (MW test)                            | 0.215          | 0.943          | 1.000          |
| rs9332108 T>C                          |                |                |                |
| Homozygotes for major allele (N=253)   | 456 (101)      | 272 (129)      | 96 (50)        |
| Heterozygotes and homozygotes for minor (variant) allele (N=28) | 451.5 (88) | 240.5 (143) | 99.5 (42) |
| P (MW test)                            | 0.715          | 0.717          | 0.469          |
| rs1057911 A>T                          |                |                |                |
| Homozygotes for major allele (N=254)   | 456.5 (101)    | 268.5 (129)    | 96.5 (50)      |
| Heterozygotes and homozygotes for minor (variant) allele (N=28) | 451.5 (88) | 240.5 (143) | 99.5 (42) |
| P (MW test)                            | 0.709          | 0.745          | 0.484          |
| rs8061994 G>A                          |                |                |                |
| Homozygotes for major allele (N=223)   | 457 (97)       | 253 (129)      | 97 (47)        |
| Heterozygotes and homozygotes for minor (variant) allele (N=57) | 450 (80) | 281 (144) | 100 (58) |
| P (MW test)                            | 0.850          | 0.983          | 0.415          |
| rs58407626 C>G                         |                |                |                |
| Homozygotes for major allele (N=265)   | 456 (95)       | 270 (128)      | 99 (49)        |
| Heterozygotes and homozygotes for minor (variant) allele (N=17) | 427 (131) | 215 (165) | 81 (44) |
| P (MW test)                            | 0.391          | 0.295          | 0.090          |

Data are shown as median and interquartile range (IQR); * P using Mann-Whitney (MW) test for differences between 2 analyzed genotypes for each SNP; ASA – acetylsalicylic acid; N – number of carriers for each genotype; MAF – minor allele frequency for each analyzed SNP in investigated cohort.
## Supplemental Table 3

Genotypes distribution (dominant model) for predefined cut-off values for CEPI-CT for PFA-100 (<165sec, <193 sec and <300sec) using exact chi-square statistics.

| Analyzed SNPs in ASA metabolism pathway | CEPI-CT (sec.) | CEPI-CT (sec.) | CEPI-CT (sec.) |
|----------------------------------------|---------------|---------------|---------------|
|                                        | >193          | ≤193          | >165          | ≤165          | 300             | <300             |
| rs28750179 G>A                         |               |               |               |
| Homozygotes [major allele] (N)         | 148           | 73            | 172           | 49            | 121             | 99               |
| Hetero-, homozygotes [minor (variant) allele] (N) | 45         | 21            | 51            | 15            | 44              | 21               |
| \(P^*\) (chi-square test)              | 0.854         | 0.924         | 0.069         |
| rs5716 G>C                             |               |               |               |
| Homozygotes [major allele] (N)         | 188           | 89            | 215           | 62            | 159             | 117              |
| Hetero-, homozygotes [minor (variant) allele] (N) | 5           | 5             | 8             | 2             | 6               | 3                |
| \(P^*\) (chi-square test)              | 0.931         | 0.799         | 0.072         |
| rs99228053 G>A                         |               |               |               |
| Homozygotes [major allele] (N)         | 190           | 93            | 220           | 63            | 163             | 118              |
| Hetero-, homozygotes [minor (variant) allele] (N) | 3           | 1             | 5             | 1             | 2               | 2                |
| \(P^*\) (chi-square test)              | 0.739         | 0.896         | 0.747         |
| rs7192210 G>A                          |               |               |               |
| Homozygotes [major allele] (N)         | 177           | 86            | 203           | 60            | 149             | 113              |
| Hetero-, homozygotes [minor (variant) allele] (N) | 4           | 3             | 1             | 1             | 5               | 3                |
| \(P^*\) (chi-square test)              | 0.462         | 0.408         | 0.751         |
| rs5713 T>C                             |               |               |               |
| Homozygotes [major allele] (N)         | 187           | 93            | 217           | 63            | 163             | 115              |
| Hetero-, homozygotes [minor (variant) allele] (N) | 6           | 1             | 6             | 1             | 2               | 5                |
| \(P^*\) (chi-square test)              | 0.292         | 0.606         | 0.112         |
| rs17863783 G>T                         |               |               |               |
| Homozygotes [major allele] (N)         | 139           | 65            | 159           | 45            | 119             | 83               |
| Hetero-, homozygotes [minor (variant) allele] (N) | 3           | 1             | 3             | 1             | 2               | 2                |
| \(P^*\) (chi-square test)              | 0.615         | 0.878         | 0.588         |
| rs6759892 T>G                           |               |               |               |
| Homozygotes [major allele] (N)         | 54            | 29            | 64            | 19            | 46              | 37               |
| Hetero-, homozygotes [minor (variant) allele] (N) | 139         | 65            | 159           | 45            | 119             | 83               |
| \(P^*\) (chi-square test)              | 0.553         | 0.370         | 0.684         |
| rs1105880 A>G                          |               |               |               |
| Homozygotes [major allele] (N)         | 67            | 36            | 77            | 26            | 58              | 45               |
| Hetero-, homozygotes [minor (variant) allele] (N) | 126         | 58            | 146           | 38            | 107             | 75               |
| \(P^*\) (chi-square test)              | 0.553         | 0.370         | 0.684         |
| rs2070959 A>G                          |               |               |               |
| Homozygotes [major allele] (N)         | 130           | 36            | 80            | 26            | 59              | 47               |
| Hetero-, homozygotes [minor (variant) allele] (N) | 123         | 58            | 143           | 38            | 106             | 73               |
| \(P^*\) (chi-square test)              | 0.738         | 0.488         | 0.557         |
| rs9332108 A>G                          |               |               |               |
| Homozygotes [major allele] (N)         | 174           | 82            | 200           | 56            | 147             | 108              |
| Hetero-, homozygotes [minor (variant) allele] (N) | 19           | 11            | 23            | 7             | 17              | 12               |
| \(P^*\) (chi-square test)              | 0.608         | 0.855         | 0.920         |
Supplemental Table 3 continued. Genotypes distribution (dominant model) for predefined cut-off values for CEPI-CT for PFA-100 (<165 sec, <193 sec and <300 sec) using exact chi-square statistics.

| Analyzed SNPs in ASA metabolism pathway | CEPI-CT (sec.) | CEPI-CT (sec.) | CEPI-CT (sec.) |
|----------------------------------------|----------------|----------------|----------------|
|                                        | >193           | ≤193           | >165           |
|                                        | 165            | 300            | <300           |
| rs1057911 A>T                          |                |                |                |
| Homozygotes [major allele] (N)         | 174            | 83             | 200            |
|                                        | 148            | 108            |                |
| Hetero-, homozygotes [minor (variant) allele] (N) | 19             | 11             | 7              |
|                                        | 17             | 12             |                |
| P* (chi-square test)                   | 0.629          | 0.886          | 0.933          |
| rs8061994 G>A                          |                |                |                |
| Homozygotes [major allele] (N)         | 155            | 72             | 179            |
|                                        | 131            | 39             |                |
| Hetero-, homozygotes [minor (variant) allele] (N) | 38             | 20             | 43             |
|                                        | 32             | 25             |                |
| P* (chi-square test)                   | 0.688          | 0.440          | 0.803          |
| rs58407626 C>G                          |                |                |                |
| Homozygotes [major allele] (N)         | 184            | 86             | 212            |
|                                        | 154            | 114            |                |
| Hetero-, homozygotes [minor (variant) allele] (N) | 10             | 7              | 11             |
|                                        | 11             | 6              |                |
| P* (chi-square test)                   | 0.426          | 0.184          | 0.557          |

N – number of carriers for each genotype; CEPI-CT – collagen/epinephrine closure time in seconds; * P using chi-square test for differences between 2 analyzed genotypes for each SNP.

impaired ASA metabolism in studied population, but not through direct association with the pharmacodynamic effect of ASA. It is also possible that some other variants, not investigated in our cohort, could influence ASA metabolism and thus modify platelet reactivity. Finally, it cannot be ruled out that the observed nominal statistically significant results did not reach statistical significance using multiple comparison correction because the number of investigated patients was too small to obtain statistically significant results for the smaller than initially assumed OR.

**Conclusions**

In summary, the results of our study failed to confirm that the selected variants in genes within the ASA metabolic pathway might contribute to platelet reactivity in a diabetic population treated with ASA. Our current results may support the hypothesis that any association between altered ASA metabolism and genetic background that may exist is likely to be weak, because the cardiovascular protection of ASA is “saturable” at daily doses of between 75 and 160 mg day, similarly to the dose range which reaches the ceiling effect in inhibiting serum TxB₂.

**Declaration of interest**

The authors state that they have no conflicts of interest.

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