Polymorphisms in GP6, PEAR1A, MRVI1, PIK3CG, JMJD1C, and SHH Genes in Patients with Unstable Angina

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Received: 16 September 2020; Accepted: 13 October 2020; Published: 15 October 2020

Abstract: Introduction: Coronary artery disease (CAD) is a significant public health problem because it is one of the major causes of death worldwide. Several studies have investigated the associations between CAD and polymorphisms in genes connected with platelet aggregation and the risk of venous thromboembolism. Aim: In this study, we examined the associations between polymorphisms in GP6 (rs1671152), PEAR1A (rs12566888), MRVI1 (rs7940646), PIK3CG (rs342286), JMJD1C (rs10761741), SHH (rs2363910), and CAD in the form of unstable angina as well as selected clinical and biochemical parameters. The study enrolled 246 patients with diagnosed unstable angina and 189 healthy controls. Results: There were no significant differences in the distribution of the studied polymorphisms between the patients with unstable angina and the controls. In patients with the GP6 rs1671152 GG genotype, we observed increased BMI values and an increased frequency of type 2 diabetes diagnosis. Conclusions: The results of this study suggest a lack of association between GP6 (rs1671152), PEAR1A (rs12566888), MRVI1 (rs7940646), PIK3CG (rs342286), JMJD1C (rs10761741), SHH (rs2363910), and unstable angina. The results indicate an association between GP6 (rs1671152) and type 2 diabetes.

Keywords: coronary artery disease; unstable angina; venous thromboembolism; platelet aggregation; polymorphism

1. Introduction

Diseases of the circulatory system, including coronary artery disease (CAD), are important public health problems because they are major causes of death worldwide. Several studies have investigated the associations between CAD and polymorphisms in genes connected with platelet aggregation and the risk of venous thromboembolism. A genome-wide association study of European and American populations identified seven loci which may be associated with platelet aggregation: JMJD1C, GP6, ADRA2A, PEAR1, SHH, PIK3CG, and MRVI1 [1]. However, the results are still controversial. CAD is a multifactorial disease that results from complex interactions between many genetic and environmental factors. Imbalance between platelets and the endothelium can cause the development of atherosclerotic lesions and cardiovascular diseases.

Glycoprotein VI (GPVI or GP6) is expressed by platelets as a receptor for the collagen [2]. The platelet membrane GPVI receptor plays an important role in coagulation processes. It has been
shown that the GPVI receptor expression is genetically determined and that polymorphisms in these genes may change the expression of GPVI receptor and influence the coagulation processes [3].

**PEAR1** is called aggregation receptor 1, and is expressed on platelets and endothelial cells as a type 1 membrane protein; it plays a role in aggregation-induced signaling. It also undergoes tyrosine phosphorylation after platelet–platelet contact [4]. Previous studies have suggested that polymorphisms in the PEAR1 gene may alter the platelet reactivity and may be associated with the development of thromboembolism and cardiovascular diseases [5].

**TRIP1 to TRIP15** genes encode thyroid hormone receptor β (TRβ)-binding proteins. Among the 15 **TRIP** genes, the human **TRIP8** gene (also known as **JMJD1C**) consists of 26 exons and is localized on chromosome 10 (10q21.3) [6]. The **JMJD1C** gene encodes a histone demethylase that regulates the synthesis of thyroid hormone and androgen receptors, and it is expressed in pluripotent cells [7–9].

**IRAG** (also known as **MRVI1**, **JEDI**, and **MEGF12**) is an inositol trisphosphate receptor-associated cGMP kinase substrate which is an endoplasmic reticulum-anchored membrane protein. A deficiency of this protein or mutation in the **IRAG** gene can impair smooth muscle relaxation and reduce the inhibition of platelet activation [10,11]. A study with mice proved that IRAG is involved in the blocking of platelet aggregation [12].

The **PIK3CG** gene is located on chromosome 7 and encodes an enzyme that phosphorylates phosphoinositides. PI3Kγ is expressed in cardiomyocytes, endothelial cells, fibroblasts, and vascular smooth muscle cells, and it plays a role in myocardial metabolism [13].

The morphogen sonic hedgehog (SHH) is a protein in humans that is encoded by the **SHH** gene on chromosome 7. Previous studies suggest that polymorphism in the SHH gene may be associated with platelet aggregation [1], and the SHH protein regulates angiogenesis and tissue regeneration by modulating the expression of multiple growth factors [14–17].

The aim of this study was to examine the associations between polymorphisms in **GP6** (rs1671152), **PEAR1A** (rs12566888), **MRVI1** (rs7940646), **PIK3CG** (rs342286), **JMJD1C** (rs10761741), and **SHH** (rs2363910) and CAD in the form of unstable angina, as well as to study selected clinical and biochemical parameters.

2. Materials and Methods

2.1. Patients

This study enrolled 246 patients (age 62.7 ± 9.9) with unstable angina that was diagnosed in the years 2017–2018. Unstable angina was diagnosed on the basis of typical clinical symptoms, ST segment or T wave changes in the electrocardiography (ECG), and >70% stenosis of at least one major coronary artery in coronary angiography. The control group consisted of 189 healthy control subjects (age 65.5 ± 11.0). The controls (n = 189) were subjects with negative findings following coronary angiography. The patients were recruited in accordance with the principles of the Declaration of Helsinki, and the study was approved by the ethics committee at Pomeranian Medical University (KB-0012/46/17), Szczecin, Poland; written informed consent was obtained from all subjects.

2.2. Genotyping

DNA was isolated from peripheral blood using the GenElute Mammalian Genomic DNA Miniprep Kit (Sigma-Aldrich, St. Louis, MO, USA) according to the manufacturer’s protocol. All the samples were genotyped in duplicates using an allelic discrimination assay on a CFX Connect Real-Time PCR Detection System (Bio-Rad, Feldkirchen, Germany) with TaqMan® probes.

2.3. Statistical Analysis

The consistency of the genotype distribution with the Hardy–Weinberg equilibrium (HWE) was assessed using the exact test. The genotype and allele distributions were compared between groups.
using chi-square test; the non-parametric Mann–Whitney U test was used to compare the clinical parameters between groups. $p < 0.05$ was considered statistically significant.

3. Results

The distributions of the studied polymorphisms were in HWE. The distribution of the studied polymorphisms in patients with unstable angina and in the control subjects is presented in Tables 1 and 2. As shown, there were no significant differences in the distribution of the studied polymorphisms between patients with unstable angina and the controls.

Additionally, we examined the associations between the studied polymorphisms and clinical parameters, such as BMI, waist circumference, total cholesterol, HDL, LDL, and triacylglycerols (Tables 3–8). We observed increased BMI values in carriers of the $GP6$ rs1671152 GG genotype and lower TG levels in carriers of the $SHH$ rs2363910 GG genotype.

Table 1. Distributions of the $PEARIA$, $MRVI1$, and $GP6$ genotypes and alleles in patients with unstable angina and controls.

| Genotype/Allele | Control Group | Unstable Angina | $p$ Value | $p$ Value $^*$ | OR (95% CI) |
|-----------------|---------------|-----------------|-----------|---------------|-------------|
| $PEARIA$        |               |                 |           |               |             |
| rs12566888      |               |                 |           |               |             |
| Genotype        | n  | %     | n  | %     |             |             |
| GG              | 152 | 80.85 | 199 | 83.97 | 0.57        |             |
| GT              | 34  | 18.09 | 37  | 15.61 |             |             |
| TT              | 2   | 1.06  | 1   | 0.42  |             |             |
| Allele          |     |       |     |       |             |             |
| G               | 338 | 89.89 | 435 | 91.77 |             |             |
| T               | 38  | 10.11 | 39  | 8.23  |             |             |
| $MRVI1$         |               |                 |           |               |             |
| rs7940646       |               |                 |           |               |             |
| Genotype        | n  | %     | n  | %     |             |             |
| CC              | 80  | 42.33 | 100 | 40.65 | 0.82        |             |
| CT              | 86  | 45.50 | 119 | 48.37 |             |             |
| TT              | 23  | 12.17 | 27  | 10.98 |             |             |
| Allele          |     |       |     |       |             |             |
| C               | 246 | 65.08 | 319 | 64.84 |             |             |
| T               | 132 | 34.92 | 173 | 35.16 |             |             |
| $GP6$           |               |                 |           |               |             |
| rs1671152       |               |                 |           |               |             |
| Genotype        | n  | %     | n  | %     |             |             |
| GG              | 157 | 83.07 | 206 | 83.74 | 0.49        |             |
| GT              | 30  | 15.87 | 34  | 13.82 |             |             |
| TT              | 2   | 1.06  | 6   | 2.44  |             |             |
| Allele          |     |       |     |       |             |             |
| G               | 344 | 91.01 | 445 | 90.65 |             |             |
| T               | 34  | 8.99  | 46  | 9.35  |             |             |

$^*$ $\chi^2$ test; Alleles: A–adenine, C–cytosine, G–guanine, T–thymine; HWE: control group $p = 1.00$, unstable angina $p = 1.00$ for $PEARIA$ rs12566888; HWE: control group $p = 1.00$, unstable angina $p = 0.40$ for $MRVI1$ rs7940646; HWE: control group $p = 0.65$, unstable angina $p = 0.011$ for $GP6$ rs1671152.
Table 2. Distributions of the PIK3CG, JMJD1C, and SHH genotypes and alleles in patients with unstable angina and controls.

|                      | Control Group | Unstable Angina | p Value * | p Value χ² | OR (95% CI) |
|----------------------|---------------|-----------------|-----------|------------|-------------|
|                      | n             | %               | n         | %          |             |
| **PIK3CG rs342286 genotype** |               |                 |           |            |             |
| AA                   | 55            | 29.26%          | 65        | 26.75%     | 0.76        |
| AG                   | 98            | 52.13%          | 127       | 52.26%     |             |
| GG                   | 35            | 18.62%          | 51        | 20.99%     |             |
|                      |               |                 |           |            | GG vs. AA   |
|                      |               |                 |           |            | 0.46        |
|                      |               |                 |           |            | 1.23 (0.70–2.16) |
|                      |               |                 |           |            | AA vs. AG   |
|                      |               |                 |           |            | 0.69        |
|                      |               |                 |           |            | 1.10 (0.70–1.71) |
|                      |               |                 |           |            | GG vs. AG   |
|                      |               |                 |           |            | 0.65        |
|                      |               |                 |           |            | 1.12 (0.68–1.86) |
| **Allele**           |               |                 |           |            |             |
| A                    | 208           | 53.32%          | 257       | 52.88%     | 0.48        |
| G                    | 168           | 44.69%          | 229       | 47.12%     |             |
| **JMJD1C rs10761741 genotype** |               |                 |           |            |             |
| GG                   | 68            | 36.36%          | 86        | 35.10%     | 0.70        |
| GT                   | 89            | 47.59%          | 112       | 45.71%     |             |
| TT                   | 30            | 16.04%          | 47        | 19.18%     |             |
|                      |               |                 |           |            | TT vs. GG   |
|                      |               |                 |           |            | 0.45        |
|                      |               |                 |           |            | 1.24 (0.71–2.16) |
|                      |               |                 |           |            | GT vs. GG   |
|                      |               |                 |           |            | 0.98        |
|                      |               |                 |           |            | 1.00 (0.65–1.52) |
|                      |               |                 |           |            | TT vs. GT   |
|                      |               |                 |           |            | 0.42        |
|                      |               |                 |           |            | 1.24 (0.73–2.13) |
| **Allele**           |               |                 |           |            |             |
| G                    | 225           | 60.16%          | 284       | 57.96%     |             |
| T                    | 149           | 39.84%          | 206       | 42.04%     |             |
| **SHH rs2363910 genotype** |               |                 |           |            |             |
| GG                   | 155           | 82.01%          | 199       | 81.22%     | 0.93        |
| GT                   | 33            | 17.46%          | 44        | 17.76%     |             |
| TT                   | 1             | 0.53%           | 2         | 0.82%      |             |
|                      |               |                 |           |            | TT vs. GG   |
|                      |               |                 |           |            | 0.72        |
|                      |               |                 |           |            | 1.55 (0.14–17.19) |
|                      |               |                 |           |            | GT vs. GG   |
|                      |               |                 |           |            | 0.88        |
|                      |               |                 |           |            | 1.04 (0.63–1.71) |
|                      |               |                 |           |            | TT vs. GT   |
|                      |               |                 |           |            | 0.74        |
|                      |               |                 |           |            | 1.50 (0.13–17.25) |
| **Allele**           |               |                 |           |            |             |
| G                    | 343           | 90.74%          | 442       | 90.20%     |             |
| T                    | 35            | 9.26%           | 48        | 9.80%      |             |

* χ² test; HWE: control group p = 0.55, unstable angina p = 0.52 for PIK3CG rs342286; HWE: control group p = 1.00, unstable angina p = 0.36 for JMJD1C rs10761741; HWE: control group p = 1.00, unstable angina p = 1.00 for SHH rs2363910.

Table 3. Associations between the clinical parameters of patients with unstable angina and the PEAR1A rs12566888 genotypes.

| Parameters | PEAR1A rs12566888 Genotype |
|------------|-----------------------------|
|            | GG                         | GT | GG+GT | n   | GT+TT | GG vs. GT+TT |
|            | Mean ± SD                  |    | Mean ± SD |    | Mean ± SD | p 6 |
| Age [years]| 199 62.8 ± 10.0            | 37 | 62.0 ± 8.5 | 236 | 62.6 ± 9.8 | 38 | 62.0 ± 8.4 | 0.64 |
| BMI [kg/m²]| 199 28.2 ± 3.9             | 37 | 28.6 ± 3.8 | 236 | 28.3 ± 3.8 | 38 | 28.5 ± 3.7 | 0.78 |
| Waist [cm] | 198 95.2 ± 10.0            | 37 | 96.5 ± 10.1 | 235 | 95.4 ± 10.0 | 38 | 96.4 ± 10.0 | 0.54 |
| CH [mg/dL] | 180 237.4 ± 63.0           | 33 | 228.1 ± 56.9 | 213 | 236.0 ± 62.1 | 34 | 228.6 ± 56.1 | 0.56 |
| HDL [mg/dL]| 144 45.4 ± 8.4             | 32 | 44.6 ± 8.7 | 176 | 45.3 ± 8.5 | 33 | 44.9 ± 8.6 | 0.52 |
| LDL [mg/dL]| 144 169.6 ± 57.7           | 32 | 162.2 ± 55.3 | 176 | 163.8 ± 57.1 | 33 | 162.5 ± 54.4 | 0.48 |
| TG [mg/dL] | 173 144.7 ± 74.3           | 33 | 149.4 ± 99.1 | 206 | 145.7 ± 78.6 | 34 | 149.3 ± 97.6 | 0.88 |

6—Mann–Whitney U test; BMI—body mass index; CH—total cholesterol in serum; HDL—high density cholesterol in serum; LDL—low density cholesterol in serum; TG—triacylglycerols in serum.
Table 4. Associations between the clinical parameters of patients with unstable angina and the MRVI1 rs7940646 genotypes.

| Parameters | MRVI1 rs7940646 Genotype |        |        |        |        |        |        |        |        |
|------------|---------------------------|--------|--------|--------|--------|--------|--------|--------|--------|
|            | CC                        | CT     | TT     | CC+CT  | n      | CT+TT  | CC vs. CT+TT | CC+CT vs. TT | CC vs. TT |
|            | n | Mean ± SD | n | Mean ± SD | n | Mean ± SD | n | Mean ± SD | n | Mean ± SD | p |        |
| Age [years]| 100 | 63.3 ± 9.8 | 119 | 62.0 ± 10.2 | 27 | 63.0 ± 9.0 | 219 | 62.6 ± 10.0 | 146 | 62.2 ± 9.9 | 0.43 | 0.78 | 0.94 |
| BMI [kg/m²]| 100 | 28.5 ± 4.4 | 119 | 28.4 ± 3.5 | 27 | 27.7 ± 3.1 | 219 | 28.4 ± 3.9 | 146 | 28.3 ± 3.4 | 0.78 | 0.39 | 0.43 |
| Waist [cm] | 99 | 95.6 ± 10.7 | 119 | 95.4 ± 9.8 | 27 | 97.0 ± 11.2 | 218 | 95.5 ± 10.2 | 146 | 95.7 ± 10.1 | 0.72 | 0.70 | 0.66 |
| CH [mg/dL]| 92 | 229.9 ± 54.9 | 108 | 239.1 ± 66.0 | 23 | 245.7 ± 69.9 | 200 | 234.9 ± 61.2 | 131 | 240.3 ± 66.5 | 0.37 | 0.43 | 0.33 |
| LDL [mg/dL]| 80 | 45.1 ± 8.7 | 86 | 45.6 ± 8.1 | 17 | 45.0 ± 9.3 | 166 | 45.3 ± 8.3 | 103 | 45.5 ± 8.2 | 0.79 | 0.94 | 0.98 |
| TG [mg/dL]| 91 | 143.5 ± 79.0 | 103 | 148.9 ± 79.1 | 22 | 136.3 ± 61.2 | 194 | 146.4 ± 78.9 | 125 | 146.7 ± 76.2 | 0.69 | 0.69 | 0.84 |

&—Mann–Whitney U test; BMI—body mass index; CH—total cholesterol in serum; HDL—high density cholesterol in serum; LDL—low density cholesterol in serum; TG—triacylglycerols in serum.

Table 5. Associations between the clinical parameters of patients with unstable angina and the GP6 rs1671152 genotypes.

| Parameters | GP6 rs1671152 Genotype |        |        |        |        |        |        |        |        |
|------------|-------------------------|--------|--------|--------|--------|--------|--------|--------|--------|
|            | GG                      | GT     | TT     | GG+GT  | n      | GT+TT  | GG vs. GT+TT | GG+GT vs. TT | GG vs. TT |
|            | n | Mean ± SD | n | Mean ± SD | n | Mean ± SD | n | Mean ± SD | n | Mean ± SD | p |        |
| Age [years]| 206 | 62.9 ± 9.9 | 34 | 61.1 ± 10.0 | 6 | 63.7 ± 8.2 | 240 | 62.6 ± 9.9 | 40 | 61.5 ± 9.7 | 0.32 | 0.79 | 0.85 |
| BMI [kg/m²]| 206 | 28.6 ± 3.8 | 34 | 27.2 ± 3.7 | 6 | 26.3 ± 4.1 | 240 | 28.4 ± 3.8 | 40 | 27.1 ± 3.8 | 0.021 | 0.13 | 0.10 |
| Waist [cm] | 206 | 96.0 ± 10.3 | 33 | 94.1 ± 10.7 | 6 | 91.2 ± 11.0 | 239 | 95.8 ± 10.3 | 39 | 93.6 ± 10.7 | 0.21 | 0.21 | 0.19 |
| CH [mg/dL]| 187 | 234.9 ± 62.5 | 30 | 241.3 ± 62.7 | 6 | 246.0 ± 50.4 | 247 | 235.7 ± 62.4 | 36 | 242.1 ± 60.2 | 0.34 | 0.43 | 0.41 |
| HDL [mg/dL]| 153 | 45.3 ± 8.5 | 25 | 45.3 ± 8.2 | 5 | 45.8 ± 6.0 | 178 | 45.3 ± 8.5 | 30 | 45.4 ± 7.7 | 0.80 | 0.73 | 0.71 |
| LDL [mg/dL]| 153 | 167.1 ± 57.8 | 25 | 173.1 ± 55.8 | 5 | 193.4 ± 38.0 | 178 | 167.9 ± 57.4 | 30 | 176.5 ± 53.2 | 0.21 | 0.19 | 0.18 |
| TG [mg/dL]| 181 | 145.2 ± 77.9 | 29 | 154.2 ± 77.2 | 6 | 107.5 ± 45.5 | 210 | 146.4 ± 77.7 | 35 | 146.2 ± 74.4 | 0.93 | 0.17 | 0.18 |

&—Mann–Whitney U test; BMI—body mass index; CH—total cholesterol in serum; HDL—high density cholesterol in serum; LDL—low density cholesterol in serum; TG—triacylglycerols in serum.
Table 6. Associations between the clinical parameters of patients with unstable angina and the PIK3CG genotypes.

| Parameters | PIK3CG rs342286 Genotype |   |   |   |   |   |   |   |   |
|------------|--------------------------|---|---|---|---|---|---|---|---|
|            | AA                       | AG | GG | AA+AG | n | AG+GG | AA+AG vs. AG+GG | AA vs. GG |
| n          | Mean ± SD                | n | Mean ± SD | n | Mean ± SD | n | Mean ± SD | p |
| Age [years]| 65                       | 64.2 ± 9.1 | 127 | 62.5 ± 10.0 | 51 | 61.1 ± 10.5 | 192 | 63.1 ± 9.7 | 1.16  |
| BMI [kg/m²]| 65                       | 28.0 ± 3.4 | 127 | 28.5 ± 3.9 | 51 | 28.1 ± 4.4 | 192 | 28.4 ± 3.7 | 0.73  |
| Waist [cm] | 64                       | 95.6 ± 9.7 | 127 | 95.5 ± 10.7 | 51 | 95.7 ± 10.5 | 191 | 95.3 ± 10.3 | 0.97  |
| CH [mg/dL] | 60                       | 238.8 ± 70.6 | 113 | 234.0 ± 59.4 | 48 | 237.3 ± 58.8 | 173 | 235.6 ± 63.3 | 0.84  |
| LDL [mg/dL]| 45                       | 47.7 ± 8.2 | 94  | 44.4 ± 8.7 | 42 | 44.5 ± 7.6 | 139 | 45.5 ± 8.7 | 0.84  |
| TG [mg/dL] | 58                       | 143.2 ± 74.2 | 110 | 148.8 ± 87.0 | 46 | 139.1 ± 55.3 | 168 | 146.9 ± 82.6 | 0.70  |

&Mann–Whitney U test; BMI—body mass index; CH—total cholesterol in serum; HDL—high density cholesterol in serum; LDL—low density cholesterol in serum; TG—triacylglycerols in serum.

Table 7. Associations between the clinical parameters of patients with unstable angina and the JMJD1C rs10761741 genotypes.

| Parameters | JMJD1C rs10761741 Genotype |   |   |   |   |   |   |   |   |
|------------|-----------------------------|---|---|---|---|---|---|---|---|
|            | GG                         | GT | TT | GG+GT | n | GT+TT | GG vs. GT+TT | GG vs. TT |
| n          | Mean ± SD                  | n | Mean ± SD | n | Mean ± SD | n | Mean ± SD | p |
| Age [years]| 86                        | 63.7 ± 9.5 | 112 | 62.4 ± 10.4 | 47 | 61.5 ± 9.4 | 198 | 63.0 ± 10.0 | 0.20  |
| BMI [kg/m²]| 86                        | 28.2 ± 3.6 | 112 | 28.6 ± 4.2 | 47 | 27.9 ± 3.6 | 198 | 28.4 ± 3.9 | 0.74  |
| Waist [cm] | 85                        | 95.6 ± 10.2 | 112 | 95.8 ± 11.0 | 47 | 95.2 ± 9.0 | 197 | 95.7 ± 10.6 | 0.94  |
| CH [mg/dL] | 80                        | 243.0 ± 63.7 | 96  | 230.4 ± 54.9 | 46 | 236.8 ± 72.9 | 176 | 236.1 ± 59.2 | 0.15  |
| HDL [mg/dL]| 69                        | 45.5 ± 8.4 | 79  | 45.7 ± 7.9 | 35 | 44.1 ± 9.5 | 148 | 45.6 ± 8.1 | 0.74  |
| LDL [mg/dL]| 69                        | 174.2 ± 60.9 | 79  | 161.9 ± 51.4 | 35 | 172.9 ± 60.9 | 148 | 167.6 ± 56.2 | 0.37  |
| TG [mg/dL] | 77                        | 158.8 ± 96.8 | 94  | 135.4 ± 59.0 | 44 | 144.8 ± 70.9 | 171 | 146.0 ± 78.9 | 0.18  |

&Mann–Whitney U test; BMI—body mass index; CH—total cholesterol in serum; HDL—high density cholesterol in serum; LDL—low density cholesterol in serum; TG—triacylglycerols in serum.
Table 8. Associations between the clinical parameters of patients with unstable angina and the SHH rs2363910 genotypes.

| Parameters      | SHH rs2363910 Genotype |        |        |        |        |        |        |        |        |        |        |        |
|-----------------|------------------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
|                 | GG                     | GT     | TT     | GG+GT  | n      | GT+TT  | GG vs. GT+TT | GG+GT vs. TT | GG vs. TT | p<sup>6</sup> |
| Age [years]     | 199                    | 61.9 ± 9.8 | 44     | 66.1 ± 9.6 | 2       | 59.0 ± 17.0 | 243          | 62.7 ± 9.9 | 46       | 65.8 ± 9.8 | 0.029 | 0.62 | 0.66 |
| BMI [kg/m<sup>2</sup>] | 199                  | 28.4 ± 3.8 | 44     | 27.9 ± 3.8 | 2       | 29.0 ± 2.8 | 243          | 28.3 ± 3.8 | 46       | 28.0 ± 3.8 | 0.40  | 0.67 | 0.71 |
| Waist [cm]      | 198                    | 95.3 ± 10.0 | 44     | 97.0 ± 12.0 | 2       | 98.0 ± 2.8 | 242          | 95.6 ± 10.4 | 46       | 97.1 ± 11.7 | 0.54  | 0.76 | 0.74 |
| CH [mg/dL]      | 180                    | 233.9 ± 59.4 | 40     | 245.0 ± 73.3 | 2       | 271.5 ± 68.6 | 220          | 235.9 ± 62.1 | 42       | 246.2 ± 72.5 | 0.42  | 0.34 | 0.31 |
| HDL [mg/dL]     | 154                    | 45.4 ± 8.5 | 27     | 44.5 ± 8.1 | 2       | 45.5 ± 3.5 | 181          | 45.3 ± 8.4 | 29       | 44.6 ± 7.8 | 0.75  | 0.80 | 0.81 |
| LDL [mg/dL]     | 154                    | 164.7 ± 53.4 | 27     | 189.8 ± 73.1 | 2       | 182.5 ± 40.3 | 181          | 168.5 ± 57.2 | 29       | 189.3 ± 70.9 | 0.090 | 0.49 | 0.44 |
| TG [mg/dL]      | 176                    | 139.1 ± 70.5 | 37     | 172.2 ± 102.1 | 2       | 182.0 ± 39.6 | 213          | 144.9 ± 77.6 | 39       | 172.7 ± 99.6 | 0.036 | 0.21 | 0.19 |

<sup>6</sup>—Mann–Whitney U test; BMI—body mass index; CH—total cholesterol in serum; HDL—high density cholesterol in serum; LDL—low density cholesterol in serum; TG—triacylglycerols in serum.
We also examined the associations between the studied genotypes and the frequency of diagnosis of type 2 diabetes mellitus and hypertension (Tables 9 and 10). Type 2 diabetes mellitus was more frequently diagnosed in carriers of the *GP6* rs1671152 GG genotype (Table 9). There were no statistically significant associations between the studied polymorphisms and the frequency of hypertension (data not shown).

**Table 9.** Distributions of the *PEAR1A*, *MRVI1*, and *GP6* genotypes and alleles in unstable angina patients with and without diabetes mellitus (DM).

|                      | Without Diabetes Mellitus | Diabetes Mellitus | p Value | p Value | OR (95% CI) |
|----------------------|---------------------------|-------------------|---------|---------|-------------|
|                      | n  | %       | n  | %       |             |
| **PEAR1A rs12566888** genotype |               |                   |         |         |             |
| GG                   | 149 | 82.32%  | 50 | 89.29%  | 0.43       | TT+GT vs. GG | 0.21 | 0.56 (0.22–1.41) |
|                     | 31  | 17.13%  | 6  | 10.71%  |             | TT vs. GT+GG | 0.58 | 0 (-)             |
|                     | 1   | 0.55%   | 0  | 0.00%   |             | TT vs. GG    | 0.56 | 0 (-)             |
|                     |     |         |    |         |             | GT vs. GG    | 0.24 | 0.58 (0.23–1.46) |
| Allele              |     |         |    |         |             | TT vs. GT    | 0.66 | 0 (-)             |
| G                   | 329 | 90.88%  | 106| 94.64%  | T vs. G     | 0.21 | 0.56 (0.23–1.38) |
| T                   | 33  | 9.12%   | 6  | 5.36%   |             |             |         |             |
| **MRVI1 rs7940646** genotype |               |                   |         |         |             |
| CC                   | 78  | 41.94%  | 22 | 36.67%  | 0.46       | TT+CT vs. CC | 0.47 | 1.25 (0.68–2.27) |
|                     | 86  | 46.24%  | 33 | 55.00%  |             | TT vs. CT+CC | 0.45 | 0.68 (0.24–1.88) |
|                     | 22  | 11.83%  | 5  | 8.33%   |             | TT vs. CC    | 0.69 | 0.81 (0.27–2.37) |
|                     |     |         |    |         |             | CT vs. CC    | 0.33 | 1.36 (0.73–2.53) |
|                     |     |         |    |         |             | TT vs. CT    | 0.32 | 0.39 (0.21–1.69) |
| Allele              |     |         |    |         |             | T vs. C      | 0.86 | 1.04 (0.68–1.60) |
| C                   | 242 | 65.05%  | 77 | 64.17%  |             |             |         |             |
| T                   | 130 | 34.95%  | 43 | 35.83%  |             |             |         |             |
| **GP6 rs1671152** genotype |               |                   |         |         |             |
| GG                   | 149 | 80.11%  | 57 | 95.00%  | 0.022      | TT+GT vs. GG | 0.007 | 0.21 (0.06–0.71) |
|                     | 31  | 16.67%  | 3  | 5.00%   |             | TT vs. GT+GG | 0.16 | 0 (-)             |
|                     | 6   | 3.23%   | 0  | 0.00%   |             | TT vs. GG    | 0.13 | 0 (-)             |
|                     |     |         |    |         |             | GT vs. GG    | 0.019 | 0.25 (0.07–0.86) |
|                     |     |         |    |         |             | TT vs. GT    | 0.45 | 0 (-)             |
| Allele              |     |         |    |         |             | T vs. G      | 0.0030 | 0.20 (0.06–0.64) |
| G                   | 329 | 88.44%  | 117| 97.50%  |             |             |         |             |
| T                   | 43  | 11.56%  | 3  | 2.50%   |             |             |         |             |

*χ² test.*
Table 10. Distributions of the PIK3CG, JMJD1C, and SHH genotypes and alleles in unstable angina patients with and without diabetes mellitus (DM).

|                      | Without Diabetes Mellitus | Diabetes Mellitus | p Value † | p Value † | OR (95% CI) |
|----------------------|---------------------------|-------------------|-----------|-----------|-------------|
|                      | n  | %         | n   | %         |      |          |
| **PIK3CG rs342286 genotype** |               |                   |          |           |             |
| AA                   | 47 | 25.54%    | 18  | 30.51%    | 0.75 |          |
| AG                   | 98 | 53.26%    | 29  | 49.15%    |      |          |
| GG                   | 39 | 21.20%    | 12  | 20.34%    |      |          |
| Allele               |    |           |     |           |      |          |
| A                    | 192| 52.17%    | 65  | 55.08%    |      |          |
| G                    | 176| 47.83%    | 53  | 44.92%    |      |          |
| **JMJD1C rs10761741 genotype** |               |                   |          |           |             |
| GG                   | 66 | 35.48%    | 20  | 33.90%    | 0.59 |          |
| GT                   | 87 | 46.77%    | 25  | 42.37%    |      |          |
| TT                   | 33 | 17.74%    | 14  | 23.73%    |      |          |
| Allele               |    |           |     |           |      |          |
| G                    | 219| 58.87%    | 65  | 55.08%    |      |          |
| T                    | 153| 41.13%    | 53  | 44.92%    |      |          |
| **SHH rs2363910 genotype** |               |                   |          |           |             |
| GG                   | 153| 82.26%    | 46  | 77.97%    | 0.48 |          |
| GT                   | 31 | 16.67%    | 13  | 22.03%    |      |          |
| TT                   | 2  | 1.08%     | 0   | 0.00%     |      |          |
| Allele               |    |           |     |           |      |          |
| G                    | 337| 90.59%    | 105 | 88.98%    |      |          |
| T                    | 35 | 9.41%     | 13  | 11.02%    |      |          |

*χ² test.

4. Discussion

In this study, we examined the associations between polymorphisms in GP6 (rs1671152), PEAR1A (rs12566888), MRVI1 (rs7940646), PIK3CG (rs342286), JMJD1C (rs10761741), and SHH (rs2363910) and unstable angina. Our results suggest a lack of statistically significant associations. Our results showed
that type 2 diabetes mellitus was more frequently diagnosed in carriers of the \textit{GPVI} rs1671152 GG genotype. Moreover, patients with this genotype had higher BMI values.

Previous studies have indicated that platelet GPVI (GP6) plays an important role in platelet function and hyperglycemia, and diabetes may alter the expression of GPVI [18]. Studies have suggested that patients with type 2 diabetes have an increased platelet expression of GPVI, which correlates with atherothrombotic complications and the frequency of cardiovascular events in these patients [19]. Additionally, patients with acute ischemic stroke had increased plasma levels of GPVI [20].

Previous studies have suggested that the SNPs in the \textit{GPVI} gene may modulate the platelet reactivity. Postula et al. [21] investigated the effect of 27 SNPs (also, rs342286 in \textit{PIK3CG}, rs1671152 in \textit{GPVI}, and rs12566888 in \textit{PEAR1}) on the platelet reactivity in 304 Caucasian diabetic patients and indicated a significance for two SNPs in the \textit{GPVI} gene: rs1671152 and rs1613662. The authors recognized that the differences in genes associated with platelet receptors may alter the platelet reactivity and may change the efficacy of platelet inhibitors [22]. In another 12-year prospective study [23], the authors evaluated the variability of the \textit{GPVI} gene in a group of patients with sticky platelet syndrome manifested as miscarriage. The authors determined the relationship between 15 selected SNPs of the GP6 gene in the studied syndrome compared to the control participants. The haplotype analysis showed a significantly higher occurrence of haplotypes combined with rs1671152, rs2304167, rs1654416, and rs1613662 in the \textit{GPVI} gene, which results in a variant of the GPVI protein, resulting in a 4.5-fold increase in the risk of fetal loss in patients versus healthy controls. Some authors [24,25] have suggested that the mechanism involved in the defected action of GPVI has a significant effect on GPVI-mediated signal transduction through tyrosine phosphorylation in a protein named Syk. The 10-fold increase in the risk of myocardial infarction associated with the possession of the 13254CC genotype was reported in a study with 525 MI patients and 474 healthy control subjects from the UK. Only this polymorphism was selected in the study because it contributes to the resulting substitution of serine 219 by proline and influences GPVI function [26]. It would be important to analyze the \textit{GPVI} gene polymorphism in patients with only diabetes.

The increased expression of GPVI plays an important role in platelet hyperactivity in patients with cardiovascular diseases [20,27]. It has been shown that hyperglycemia and diabetes may alter the expression of GPVI [19]. Previous studies suggest that patients with diabetes have elevated levels of platelet GPVI and ROS generation [28]. The combination of high glucose levels, increased oxidative stress, elevated platelet GPVI, and platelet reactivity in patients with diabetes makes this group of patients especially exposed to the development of cardiovascular diseases. Arthur et al. indicated that hyperglycemia in monkey with experimental diabetes induces ROS generation with the engagement of GPVI. The inhibition of GPVI significantly reduced the ROS generation in diabetic monkeys [29]. These results indicate that glycemic control plays a crucial role in reducing GPVI-dependent platelet hyperreactivity.

The expression of GPVI on platelets is also altered in patients with diseases of the circulatory system [30–32]. GPVI plays an important role in the activation of circulating platelets in acute coronary syndrome, ischemic stroke, and diabetes mellitus [30–32]. The studies suggest that elevated GPVI levels may be the marker of acute coronary syndromes and ischemic stroke [30–32].

Moreover, recent studies have indicated the clinical significance of GPVI in sepsis and cancer metastasis.

Soluble GPVI is a marker of platelet activation in thrombotic conditions. Montague et al. suggested that soluble GPVI is an important platelet-specific marker for platelet activation that predicts sepsis progression and mortality in injured patients [33]. Mammadova-Bach et al. have shown that GPVI plays an important role in cancer metastasis [34]. The genetic deficiency of platelet GPVI in mice decreased the experimental and spontaneous metastasis of colon and breast cancer cells. These authors suggest that GPVI may be a promising target for antimetastatic therapies.

Several studies have examined the associations between polymorphisms in genes related to platelet reactivity with the risk of CAD. As described, the \textit{JMJD1C} gene variant (rs7896518), located in
an intron, showed an association with the platelet count and volume in European [35] and African American populations [35]. On the other hand, while some showed that rs10761741 in the JMJD1C gene was associated with epinephrine-induced platelet aggregation and with higher circulating VEGF levels in a European population [36,37], others showed that the same variant in the JMJD1C gene was not associated with higher circulating VEGF levels [38]. It is important to know the roles of platelets and VEGF in the development of atherosclerosis and arterial thrombosis [38].

The results of the meta-analysis by Johnson et al. suggest that polymorphisms in the genes PEAR1 (rs12566888), MRVI1 (rs7940646), GPVI (rs1671152), and SHH (rs2363910) may be associated with differences in platelet aggregation [1]. Epinephrine-induced platelet aggregation was associated with SNPs in PEAR1 (rs12566888), PIK3CG (rs342286), and JMJD1C (rs10761741) [39]. In a genome-wide association study of 3000 unrelated men of European origin, the authors identified MRVI1 (rs7940646) as a gene variant associated with platelet reactivity, PIK3CG (rs342286) as a gene variant associated with increased mean platelet volume, JMJD1C (rs10761741) as a gene variant associated with decreased platelet reactivity, and PEAR1 (rs12566888) as a gene variant associated with reduced ADP-induced platelet aggregation [40].

As we have already reported, the rs342286 variant of the PIK3CG gene is associated with a younger age of patients with acute coronary syndrome [41]. In a study by Appelboom et al. on patients after intracerebral hemorrhage, there was a positive association between rs342286 and hematoma volume in the prospective assessment of computed tomography scans [42].

5. Conclusions

The results of this study suggest a lack of association between GP6 (rs1671152), PEAR1A (rs12566888), MRVI1 (rs7940646), PIK3CG (rs342286), JMJD1C (rs10761741), SHH (rs2363910), and unstable angina. The results indicate a positive association between the GG genotype of the GP6 (rs1671152) polymorphism and type 2 diabetes.

Author Contributions: Conceptualization, A.P.; methodology, M.S.; software, K.S.; validation, A.P., M.S.; A.M.-S.; formal analysis, V.D., M.E.R., and K.S.; investigation, A.P., R.R.; resources, A.P.; data curation, R.R.; writing original draft preparation, A.P., V.D., M.E.R.; supervision, A.P.; project administration, A.P.; and funding acquisition, A.P. All authors have read and agreed to the published version of the manuscript.

Funding: The project was financed by the Minister of Science and Higher Education in the “Regional initiative of excellence” program, in the years 2019–2022, no. 002/RID/2018/19.

Conflicts of Interest: The authors declare no conflict of interest.

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