Glutathione Transferase P1 Polymorphism Might Be a Risk Determinant in Heart Failure

Dejan Simeunovic, Natalija Odanovic, Marija Pljesa-Ercegovac, Tanja Radic, Slavica Radovanovic, Vesna Coric, Ivan Milinkovic, Marija Matic, Tatjana Djukic, Arsen Ristic, Dijana Risimic, Petar Seferovic, Tatjana Simic, Dragan Simic, and Ana Savic-Radojevic

1 Clinic of Cardiology, Clinical Center of Serbia, 8 Kosté Todoróvića, 11000 Belgrade, Serbia
2 Faculty of Medicine, University of Belgrade, 8 Doktora Subotica, 11000 Belgrade, Serbia
3 Section of Cardiovascular Medicine, Department of Internal Medicine, Yale School of Medicine, New Haven, Connecticut, USA
4 Institute of Medical and Clinical Biochemistry, 2 Pasterova, 11000 Belgrade, Serbia
5 University Clinical Hospital Center "Dr. Dragisa Mivosevic-Dedinje", Heroja Milana Tepica 1, 11000 Belgrade, Serbia
6 Clinic of Ophthalmology, Clinical Center of Serbia, 2 Pasterova, 11000 Belgrade, Serbia

Correspondence should be addressed to Dragan Simic; dvsimic@yahoo.com and Ana Savic-Radojevic; ana.savic-radojevic@med.bg.ac.rs

Received 4 February 2019; Revised 18 April 2019; Accepted 7 May 2019; Published 2 June 2019

Guest Editor: Agata M. Bielecka-Dabrowa

Disrupted redox balance in heart failure (HF) might contribute to impairment of cardiac function, by oxidative damage, or by regulation of cell signaling. The role of polymorphism in glutathione transferases (GSTs), involved both in antioxidant defense and in regulation of apoptotic signaling pathways in HF, has been proposed. We aimed to determine whether GST genotypes exhibit differential risk effects between coronary artery disease (CAD) and idiopathic dilated cardiomyopathy (IDC) in HF patients. GSTA1, GSTM1, GSTP1, and GSTT1 genotypes were determined in 194 HF patients (109 CAD, 85 IDC) and 274 age- and gender-matched controls. No significant association was found for GSTA1, GSTM1, and GSTT1 genotypes with HF occurrence due to either CAD or IDC. However, carriers of at least one variant GSTP1∗Val (rs1695) allele were at 1.7-fold increased HF risk than GSTP1∗Ile/Ile carriers (p = 0.031), which was higher when combined with the variant GSTA1∗B allele (OR = 2.2, p = 0.034). In HF patients stratified based on the underlying cause of disease, an even stronger association was observed in HF risk than GSTP1∗Ile/Ile carriers (p = 0.031), which was higher when combined with the variant GSTA1∗B allele (OR = 2.2, p = 0.034). In HF patients stratified based on the underlying cause of disease, an even stronger association was observed in HF patients due to CAD, who were carriers of a combined GSTP1(rs1695)/GSTA1 “risk-associated” genotype (OR = 2.8, p = 0.033) or a combined GSTP1∗Ile/Val+Val/Val (rs1695)/GSTP1∗Ala/Val+∗ValVal (rs1138272) genotype (OR = 2.1, p = 0.056). Moreover, these patients exhibited significantly decreased left ventricular end-systolic diameter compared to GSTA1∗AA/GSTP1∗IleIle carriers (p = 0.021). Higher values of ICAM-1 were found in carriers of the GSTP1∗IleVal+∗ValVal (rs1695) (p = 0.041) genotype, whereas higher TNFα was determined in carriers of the GSTP1∗AlaVal+∗ValVal genotype (rs1138272) (p = 0.041). In conclusion, GSTP1 polymorphic variants may determine individual susceptibility to oxidative stress, inflammation, and endothelial dysfunction in HF.

1. Introduction

For more than a decade, it has been suggested that a complex interplay between oxidative stress and chronic inflammation represents one of the underlying mechanisms of gradual cardiac depression in heart failure (HF) [1–3]. Oxidative stress in HF is believed to be a consequence of increased circulating neurohormones and hemodynamic disorder, as well as inflammation and decreased oxygen delivery. On the other hand, disturbed redox balance in patients with HF might contribute to further impairment of cardiac function, either by oxidative damage to vital cellular molecules or by affecting...
cell signaling involved in cell survival and death [4]. There is overwhelming evidence for the presence of oxidative stress in all phases of HF in animal models and humans [5, 6]. Regarding the mechanisms of oxidative stress in HF, both enhanced free radical production and diminished antioxidative defense are involved in the occurrence and progression of HF [5]. It is important to note that increased free radical production and inflammation are involved in cardiomyocyte apoptosis and progression of HF. Continuous release of free radicals in response to angiotensin II and catecholamines has also been found to take part in cardiac hypertrophy. Additionally, structural changes and activation of metalloproteinases are also dependent on free radicals produced in the course of fibroblast to myofibroblast transformation. Taken together, all these free radical-dependent processes contribute to the occurrence of end-stage HF [5]. Several biomarkers of oxidative distress, such as isoprostanes, malondialdehyde, uric acid, and protein carbonyl groups, have been shown to be elevated in different stages of HF [7, 8].

In addition to this well-established link, recent findings on the adverse effect of chemical and pollutant exposure to heart disease [9, 10] put special emphasis on the role of genetic polymorphisms of enzymes involved in detoxification of xenobiotics and antioxidant defense in the HF syndrome [11]. Members of the glutathione transferase (GST) enzyme superfamily belong to phase II detoxification enzymes but are also involved in regulation of the cellular redox state through different antioxidant catalytic and noncatalytic roles [12]. Moreover, almost all members of the GST family exhibit genetic polymorphisms, which can result in a complete lack or lowering of enzyme activity [13]. Considering the fact that HF represents a multifactorial, polygenic syndrome, the role of oxidative stress and consequently polymorphic expression of GSTs may have a different impact, especially regarding the specific cause of heart failure. In coronary artery disease (CAD) as the most common etiology of heart failure in industrialized countries, genetic epidemiologic studies mostly investigated the association of common GSTA1, GSTM1, GSTT1, and GSTP1 polymorphisms with disease risk [14–16]. Among them, the most attention was focused on the investigation of GSTM1 and GSTT1 deletion polymorphisms [17], considering the fact that the homozygous deletions of these genes result in a complete lack of enzymatic activity and thus diminish detoxification capacity [18]. Based on the important role of the GSTM1 enzyme in detoxifying benzodiol epoxide, present in tobacco smoke and environmental pollution, it could be speculated that carriers of the GSTM1-null genotype could have increased risk of CAD, particularly in smokers. Until now, the results on the independent effect of the GSTM1-null genotype on increased susceptibility to CAD are still being debated [14, 17]. On the other hand, the recent meta-analysis involving 47596 subjects showed that the GSTM1-null genotype in association with smoking increases the risk for CAD [19]. Moreover, correlation between the GSTM1-null genotype and indices of inflammation and oxidative stress has been demonstrated in CAD. Thus, higher CRP and lower total antioxidant capacity have been observed in CAD patients lacking GSTM1 than those with an active GSTM1 enzyme [20]. With regard to the GSTT1-null genotype, only few studies revealed that the GSTT1-null genotype carries higher risk for HF development [14, 17]. Two genetic variants in the GSTP1 gene, the GSTP1*G allele (rs1695) coding for protein in which amino acid isoleucine (Ile) is substituted with valine (Val) at position 105 and the GSTP1* allele (rs1138272) in which alanine (Ala) is substituted with (Val) at position 114, have been shown to confer altered catalytic and noncatalytic activity, whereas the GSTA1*B allele (rs3957356) is associated with the lower expression of GSTA1 than that of the common GSTA1*A allele. It seems reasonable to assume that GSTA1- or GSTP1-variant genotypes also might contribute to the endogenous predisposition to oxidative damage in the setting of disrupted redox balance in HF patients due to CAD. However, the results of association of GSTP1 and GSTA1 polymorphisms with risk for CAD are still inconsistent [14, 21]. Interestingly, in idiopathic dilated cardiomyopathy (IDC), as a rare entity of HF syndrome, the effect of genetic polymorphisms of these enzymes has still not been investigated.

Having all that in mind, we conducted a pilot case-control study consisting of patients with HF due to coronary artery disease (CAD) or idiopathic dilated cardiomyopathy (IDC) in order to compare the distribution of common GST genotypes and the differential risk effect between these two entities.

2. Materials and Methods

2.1. Subjects. A total of 194 patients (51 women and 143 men, all Caucasian) with HF were enrolled in the study. We included two kinds of patients in the study: 109 of those with HF due to coronary artery disease (CAD) and 85 of those with heart failure due to idiopathic dilated cardiomyopathy (IDC). All patients were recruited between 2008 and 2012 from the Medical Center “Bezanijska Kosa” and the Clinical Center of Serbia, during the dispensary checkups. Diagnosis of HF was based on the patient’s history, physical examination, electrocardiography, chest X-ray, echocardiography, and coronary angiography. Distribution of the New York Heart Association (NYHA) stage was indicated for HF patients (Table 1). Major inclusion criteria were the left ventricular ejection fraction < 45% and stable HF over the two weeks prior to enrollment. For the CAD subpopulation, the inclusion criterion was evidence of CAD on angiography. For the IDC subpopulation, major inclusion criteria were the absence of CAD on coronary angiography and the evidence of chamber dilation. Patients with congenital, acquired valvular, or pericardial abnormalities were excluded from the study. Our case-control study also included a total of 274 individuals in the control group. The study was approved by the ethics committee of the Clinical Center of Serbia (470/XII-9 from 29/12/2008), and all study participants signed an informed consent.

2.2. GST Genotyping. Genomic DNA was isolated from whole blood using the QIAGEN QIAamp kit (QIAGEN Inc., Chatsworth, CA). GSTA1 (-69C>T) and GSTP1 (Ile105Val, rs1695) were examined by the polymerase chain reaction-
restriction fragment length polymorphism (PCR-RFLP) method [22, 23], whereas the GSTM1/GSTT1 were determined by the PCR method [24]. The GSTP1 (Ala114Val, rs1138272) polymorphism was determined by qPCR (Applied Biosystems) only in CAD patients using an Applied Biosystems TaqMan Drug Metabolism Genotyping Assay (ID C_1049615_20). The primer sequences, PCR conditions, restriction enzymes used, and respective restriction conditions, as well as fragment lengths after electrophoresis on 2% agarose gel, can be found in Table 2.

2.3. Determination of Parameters of Inflammation, Oxidative Stress, and Endothelial Dysfunction in Plasma/Serum. Malondialdehyde (MDA) was determined spectrophotometrically (BIOXYTECH LPO-586 kit; OxIS Research, Portland, OR, USA). The results were expressed in μmol/L. Serum levels of hs-CRP were determined using a commercially available kit. Commercially available ELISA kits for TNFα, ICAM-1, VCAM-1 (Bender MedSystems, GmbH, Austria) were used for the measurement of those inflammatory markers in plasma/serum samples collected from each patient.

2.4. Noninvasive Assessment of Endothelium-Dependent and Endothelium-Independent Flow-Mediated Dilation (FMD) of the Brachial Artery. Endothelium-dependent and endothelium-independent FMD of the brachial artery was assessed by a 13.0 MHz linear array transducer (Vivid 7, GE Medical Systems, Little Chalfont, England, UK) as previously published.

2.5. Statistical Analysis. In descriptive statistics, we summarized all continuous variables by means ± standard deviations (SD). Differences in investigated parameters were assessed by using analysis of variance (ANOVA) and Student’s t-test for continuous variables and χ² for categorical variables. The associations between the genotypes and HF risk were calculated by using logistic regression to compute odds ratios (ORs) and corresponding 95% confidence intervals (CIs), adjusted according to age, gender, smoking, hypertension, and diabetes as potential confounding factors. Haplotype analysis of GSTP1 SNPs was examined using the SNPStats. In order to demonstrate the validity of our data, a positive control was introduced by assessing the well-established association between GSTM1 deletion polymorphism in smokers and the risk of bladder cancer [25, 26]. For this purpose, an adjusted OR to age, gender, and BMI was calculated. For the data with a nonnormal distribution, we used the Mann-Whitney rank-sum test for between-two-group comparisons. Two-tailed p values of <0.05 were considered significant. Data were analyzed using the Statistical Package for the Social Sciences (SPSS) (version 17.0, Chicago, IL).

3. Results

3.1. General Characteristics of Patients. The characteristics of the whole group of HF patients, as well as patients stratified to underlying disease due to IDC and CAD, along with the characteristics of control participants are shown in Table 1. While the average age of both the control group and the HF patients is around 55 years, patients with IDC appear to be 6 years younger on average (mean age = 49.0) and thus statistically differ from the remaining two groups, which is consistent with findings in the literature [27, 28]. The occurrence of diabetes and hypertension was significantly higher in patients and patient subpopulations compared to controls.

| Table 1: Selected characteristics of patients with HF and controls. |
|-------------------------|-----------------|-----------------|-----------------|-----------------|
|                         | Controls        | HF              | IDC             | CAD             |
| Age (years) ± SDa       | 55.8 ± 10.9     | 54.3 ± 10.8     | 49.0 ± 13.6*    | 58.7 ± 4.3     |
| Genderb                 |                 |                 |                 |                 |
| Females (%)             | 90 (33)         | 51 (26)         | 15 (18)*        | 36 (33)         |
| Males (%)               | 184 (67)        | 143 (74)        | 70 (82)*        | 73 (67)         |
| Diabetesb               |                 |                 |                 |                 |
| Yes (n (%))             | 25 (9)          | 66 (35)*        | 25 (30)*        | 41 (38)*        |
| No (n (%))              | 249 (91)        | 123 (65)*       | 57 (70)*        | 66 (62)*        |
| Hypertensionb           |                 |                 |                 |                 |
| Yes (n (%))             | 67 (26)         | 82 (52)*        | 16 (19)         | 68 (78)*        |
| No (n (%))              | 191 (74)        | 77 (48)*        | 69 (81)         | 19 (22)*        |
| Smoking statusb         |                 |                 |                 |                 |
| Smokers (n (%))         | 138 (52)        | 99 (57)         | 42 (51)         | 57 (61)         |
| Nonsmokers (n (%))      | 126 (48)        | 76 (43)         | 40 (49)         | 36 (39)         |
| NYHA                    |                 |                 |                 |                 |
| II                      | 124 (64)        | 54 (64)         | 71 (65)         |                 |
| III                     | 48 (25)         | 20 (24)         | 28 (26)         |                 |
| IV                      | 18 (9)          | 8 (9)           | 10 (9)          |                 |

HF: heart failure; IDC: idiopathic dilated cardiomyopathy; CAD: coronary artery disease; SD: standard deviation; NYHA: New York Heart Association functional classification of heart failure. aStudent’s t-test; bχ² test; *statistically significant in comparison to controls (p < 0.05).
Table 2: PCR-RFLP: primer sequences, PCR conditions, restriction enzymes, and fragment lengths.

| Polymorphism | Primer sequences | PCR protocol | Gel electrophoresis results |
|--------------|-----------------|--------------|-----------------------------|
| GSTA1*C-69T  | F, 5'-GCATCAGCTGGCCTTCA-3'  
R, 5'-AAAGCCTGTCAGGTCTCG-3' | Denature: 94°C for 3 min  
Followed by 94°C for 30 s  
Annealing: 56°C for 30 s  
Extension: 72°C for 30 s  
#cycles: 30  
Final extension: 72°C for 10 min | Eam104I incubation at 37°C overnight  
*GSTA1*CC: 400 bp  
*GSTA1*CT: 400 bp, 308 bp, and 92 bp  
*GSTA1*TT: 308 bp and 92 bp |
| GSTP1*Ile105Val | F, 5'-ACCCCAAGGCTCTTATGGGAA-3'  
R, 5'-TGAGGGCACAAGAGCGCTTCT-3' | Denature: 95°C for 10 min  
Followed by 94°C for 30 s  
Annealing: 59°C for 30 s  
Extension: 72°C for 30 s  
#cycles: 29  
Final extension: 72°C for 10 min | Alw26I incubation at 37°C overnight  
*GSTP1*Ile/Ile: 176 bp  
*GSTP1*Ile/Val: 176 bp, 91 bp, and 85 bp  
*GSTP1*Val/Val: 91 bp and 85 bp |
| GSTM1        | F, 5'-GAACCTCCTGAAAGCTAAGCG-3'  
R, 5'-GTTGGGCTCAATACGGTGGG-3' | Multiplex PCR:  
Denature: 94°C for 3 min  
Followed by 94°C for 30 s  
Annealing: 59°C for 30 s  
Extension: 72°C for 45 s  
#cycles: 30  
Final extension: 72°C for 4 min | *GSTM1*active: 215 bp band  
*GSTM1>null: absent band |
| GSTT1        | F, 5'-TTCCTAAGTGCCTCAGCTCCT-3'  
R, 5'-TCACGGGATCATGGCCAGGCA-3' |  
| CYP1A1       | F, 5'-GAACGTGCACTT CAGCTGCT-3'  
R, 5'-CAGCTGCATTG GAAGTGCTC-3' | Successful PCR reaction: 312 bp band  
Unsuccessful PCR reaction: absent band |
Gender and smoking status did not differ significantly between HF patients and controls.

3.2. Distribution of GST Genotypes. The distribution of GST genotypes in all HF patients and controls is shown in Table 3. The frequencies of the GSTA1, GSTP1, GSTM1, and GSTT1 genotypes are in accordance with the reported values in the literature. In order to fully estimate the role of GST genotypes in HF development, we performed haplotype analysis generating four GSTP1 haplotypes: wild-type GSTP1A (Ile105/Ala114), GSTP1B (Val105/Ala114), GSTP1C (Val105/Val114), and GSTP1D (Ile105/Val114). Haplotype analysis confirmed small yet nonsignificant risk for CAD-related HF development in the case of the GSTP1B haplotype (OR = 0.9; 95%CI = 0.3–2.5; p = 0.056; Table 5). On the other hand, GSTP1C and GSTP1D haplotypes exhibited lower risk towards CAD-related HF development (OR = 0.5, 95%CI = 0.1–0.9; p = 0.049, and OR = 0.6, 95%CI = 0.1–3.2, p = 0.056, respectively; Table 5).

3.3. Risk-Associated GST Genotypes in relation to the Parameters of Oxidative Stress, Inflammation, and Endothelial Dysfunction in the CAD-Related HF Subgroup. Plasma levels of MDA, end product of lipid peroxidation,
Table 4

(a) Distribution of GSTA1, GSTP1, GSTM1, and GSTT1 genotypes in HF patients due to CAD and controls

| Genotype                     | Controls (n (%)) | Patients (n (%)) | OR (95% CI)\(^a\) | \(p\)  |
|------------------------------|------------------|------------------|--------------------|-------|
| **GSTA1**                    |                  |                  |                    |       |
| \(\ast\)A/A                  | 112 (41)         | 38 (35)          | 1.0\(^b\)          |       |
| \(\ast\)A/B+B+B              | 162 (59)         | 70 (65)          | 1.8 (0.9-3.5)      | 0.075 |
| **GSTM1**                    |                  |                  |                    |       |
| Active                       | 137 (50)         | 53 (49)          | 1.0\(^b\)          |       |
| Null                         | 137 (50)         | 56 (51)          | 1.1 (0.6-2.0)      | 0.749 |
| **GSTT1**                    |                  |                  |                    |       |
| Active                       | 203 (74)         | 82 (75)          | 1.0\(^b\)          |       |
| Null                         | 71 (26)          | 27 (25)          | 1.0 (0.5-2.0)      | 0.935 |
| **GSTP1**                    |                  |                  |                    |       |
| \(\ast\)Ile/Ile              | 115 (42)         | 39 (37)          | 1.0\(^b\)          |       |
| \(\ast\)Ile/Val+\ast Val/Val| 159 (58)         | 68 (63)          | 1.9 (1.0-3.6)      | 0.056 |
| **Combined GSTA1/GSTP1**     |                  |                  |                    |       |
| \(\ast\)AA/\ast IleIle       | 54 (20)          | 16 (15)          | 1.0\(^b\)          |       |
| \(\ast\)AA/\ast IleVal+\ast Val/Val | 58 (21) | 22 (21)          | 1.4 (0.5-4.1)      | 0.539 |
| \(\ast\)AB++\ast Ala/\ast IleVal+\ast Val/Val | 61 (22) | 23 (22)          | 1.4 (0.5-4.0)      | 0.560 |
| **Combined GSTA1/GSTP1 (rs1138272)** |                  |                  |                    |       |
| \(\ast\)AA/\ast Ala/\ast Ala | 28 (11)          | 9 (9)            | 1.0\(^b\)          |       |
| \(\ast\)AA/\ast AlaVal+\ast Val/Val | 84 (33) | 29 (28)          | 1.6 (0.4-5.4)      | 0.490 |
| \(\ast\)AB++\ast Ala/\ast Ala | 24 (9)           | 7 (7)            | 0.9 (0.2-3.4)      | 0.906 |
| \(\ast\)AB+\ast AlaVal++\ast Val/Val | 121 (47) | 60 (57)          | 2.1 (1.0-4.5)      | 0.056 |

(b) Distribution of GSTA1, GSTP1, GSTM1, and GSTT1 genotypes in IDC patients and controls

| Genotype                     | Controls (n (%)) | Patients (n (%)) | OR (95% CI)\(^a\) | \(p\)  |
|------------------------------|------------------|------------------|--------------------|-------|
| **GSTA1**                    |                  |                  |                    |       |
| \(\ast\)A/A                  | 112 (41)         | 30 (35)          | 1.0\(^b\)          |       |
| \(\ast\)A/B+B+B              | 162 (59)         | 55 (65)          | 1.2 (0.6-2.2)      | 0.660 |
| **GSTM1**                    |                  |                  |                    |       |
| Active                       | 137 (50)         | 39 (46)          | 1.0\(^b\)          |       |
| Null                         | 137 (50)         | 46 (54)          | 1.2 (0.6-2.2)      | 0.620 |
| **GSTT1**                    |                  |                  |                    |       |
| Active                       | 203 (74)         | 64 (75)          | 1.0\(^b\)          |       |
| Null                         | 71 (26)          | 21 (25)          | 0.9 (0.4-1.9)      | 0.854 |
| **Combined GSTA1/GSTP1**     |                  |                  |                    |       |
| \(\ast\)AA/\ast IleIle       | 54 (20)          | 13 (15)          | 1.0\(^b\)          |       |
| \(\ast\)AA/\ast IleVal+\ast Val/Val | 58 (21) | 17 (20)          | 1.3 (0.5-3.6)      | 0.618 |
| \(\ast\)AB++\ast BB/\ast IleIle | 61 (22) | 16 (19)          | 1.0 (0.4-2.9)      | 0.924 |
| \(\ast\)AB+\ast BB/\ast IleVal++\ast Val/Val | 101 (37) | 39 (46)          | 1.5 (0.6-3.8)      | 0.348 |

CI: confidence interval; OR: odds ratio. \(^a\)Logistic regression to compute odds ratios adjusted for gender, age, smoking, hypertension, and diabetes. \(^b\)Reference group. *Statistically significant in comparison with the reference genotype (\(p < 0.05\)).
Table 5: Haplotype analysis of GSTP1 polymorphisms in CAD-related HF patients and controls.

| Haplotype | GSTP1 rs4925 | GSTP1 rs156697 | Controls (%) | CAD patients (%) | OR (95% CI) | p |
|-----------|--------------|----------------|--------------|------------------|-------------|---|
| GSTP1*A   | *A           | *A             | 58           | 59               | 1.0⁷        |   |
| GSTP1*B   | *G           | *C             | 33           | 34               | 1.4 (0.9-2.3)| 0.17 |
| GSTP1*C   | *G           | *T             | 4            | 4                | 0.5 (0.1-3.9)| 0.49 |
| GSTP1*D   | *A           | *T             | 4            | 3                | 0.6 (0.1-3.0)| 0.56 |

CI: confidence interval; OR: odds ratio. SNPSstats was used for haplotype analysis; ⁷Logistic regression to compute odds ratios adjusted for gender, age, smoking, hypertension, and diabetes. ⁸Reference group.

Table 6: Markers of oxidative stress, inflammation, and endothelial dysfunction in CAD-related HF patients stratified according to GST genotypes.

| Genotype    | MDA (μmol/L) | hs-CRP (mg/L) | TNFα (pg/mL) | ICAM-1 (ng/L) | VCAM-1 (ng/L) |
|-------------|--------------|---------------|--------------|---------------|---------------|
| GSTA1       |              |               |              |               |               |
| *A/A        | 7.53 ± 0.86  | 2.12 (0.49-43.51) | 2.25 (0.15-47.63) | 375.90 (206.43-696.91) | 1113.12 (563.85-3697.45) |
| *A/B+B/B    | 7.57 ± 0.73  | 2.14 (0.15-45.00) | 2.25 (0.22-29.76) | 380.98 (264.20-691.04) | 1063.23 (144.45-7429.55) |
| GSTP1 (rs1695) |              |               |              |               |               |
| *Ile/Ile    | 7.53 ± 0.87  | 1.91 (0.38-35.68) | 2.15 (0.33-11.12) | 363.38 (206.43-553.84) | 1152.30 (563.85-1793.90) |
| *Ile/Val+*Val/Val | 7.58 ± 0.72  | 2.29 (0.15-45.00) | 2.34 (0.15-47.63) | 390.10 (255.12-696.91) | 1095.20 (144.45-7429.55) |
| P           | 0.745        | 0.382         | 0.557        | 0.041         | 0.882         |
| GSTP1 (rs1138272) |          |               |              |               |               |
| *Ala/Ala    | 7.55 ± 0.79  | 2.14 (0.15-45.00) | 2.21 (0.15-11.12) | 381.25 (206.43-696.91) | 1109.25 (144.45-7429.55) |
| *Ala/Val+*Val/Val | 7.72 ± 0.76  | 2.08 (0.93-43.51) | 4.69 (0.63-47.63) | 351.35 (275.60-568.10) | 1040.95 (759.25-1871.55) |
| p           | 0.436        | 0.777         | 0.045        | 0.152         | 0.899         |

MDA: malondialdehyde; hs-CRP: high-sensitivity C-reactive protein; TNFα: tumor necrosis factor-alpha; ICAM-1: intercellular adhesion molecule-1; VCAM-1: vascular cell adhesion molecule-1. ⁷Student t-test was used. ⁸Mann-Whitney test was used. *Statistically significant at p < 0.05.

and established marker of lipid oxidative damage were analyzed in CAD-related HF patients stratified according to polymorphism in GSTA1 and GSTP1 antioxidant enzymes (Table 6). No significant difference was observed in MDA levels between carriers of either GSTA1 or GSTP1 (rs1695 and rs1138272) genotypes in the CAD subgroup. Moreover, inflammatory markers TNFα and hs-CRP together with biochemical markers of endothelial dysfunction, ICAM-1 and VCAM-1, were also stratified according to GST risk genotypes. The results have shown that higher values of ICAM-1 were found in carriers of GSTP1*IleVal+*Val/Val (rs1695) (p = 0.041), whereas higher TNFα was present in carriers of GSTP1*AlaVal+*Val/Val (rs1138272) (p = 0.041) (Table 6).

3.4. The Association of GSTP1 Genotype with the Indices of HF Severity. The role of GSTP1 polymorphism was further analyzed regarding parameters related to the severity of HF. The dimensions of the left ventricle after systole and diastole (LVEDD and LVEDD), along with NO-dependent and NO-independent vasodilation of the brachial artery in CAD patients with different GSTP1 genotypes, are shown in Table 7. The end-systolic (LVEDS) and end-diastolic (LVEDD) diameters of the left ventricle did not differ significantly between patients with different GSTP1 genotypes (rs1695 and rs1138272). Likewise, the degree of endothelium-dependent NO-mediated vasodilation and endothelium-independent nitroglycerin- (NTG-) mediated vasodilation of the brachial artery was similar between CAD-related HF patients with either the GSTP1 wild-type genotype and carriers of at least one variant GSTP1 allele. When we analyzed these parameters in CAD-related HF patients stratified according to combined GSTA1/GSTP1 (rs1695) genotypes (Table 7), only carriers of variant GSTA1*B/GSTP1*Val (rs1695) alleles had significantly decreased LVEDS compared to individuals with GSTA1*AA/GSTP1*Ile (p = 0.021).

LVEDD: left ventricular end-diastolic diameter; LVEDS: left ventricular end-systolic diameter; FMD-NO: endothelium-dependent NO-mediated vasodilation; FMD-NTG: endothelium-independent NTG-mediated vasodilation. ⁷Student t-test was used for testing differences in LVEDD and LVEDS, for each group compared to the reference group (GSTP1*Ile/Ile or combined GSTA1/GSTP1*AA/*Ile/Ile, GSTA1/GSTP1*AA/*Ala/Ile, GSTA1/GSTP1*AA/*Ala/Ala). ⁸Mann-Whitney test was used for testing differences in FMD-NO and FMD-NTG, for each group compared to the reference group (GSTP1*Ile/Ile or combined GSTA1/GSTP1*AA/*Ile/Ile, GSTA1/GSTP1*AA/*Ala/Ile, GSTA1/GSTP1*AA/*Ala/Ala). *Statistically significant at p < 0.05.
Based on different roles of GSTs and considering the fact that in the setting of heart failure the disturbances of redox regulation can contribute to disease progression, in this study, we investigated the effect of common GST polymorphisms regarding specific HF entities. Among tested GST polymorphisms, only the variant GSTP1*Val allele has shown a significant association with HF, regardless of the specific cause. This HF risk conferred by GSTP1 polymorphism was even higher when combined with the variant GSTA1*Val allele. In HF patients stratified according to the underlying cause of the disease, even more potentiated association was observed in HF patients due to CAD, while in those due to idiopathic cardiomyopathy, despite the evident trend, this association was not confirmed.

In our study, we found no significant association for individual GSTM1 and GSTT1 polymorphisms with the occurrence of HF due to either CAD or IDC. Our results are in concordance with the study of Norskov et al., who conducted a comprehensive analysis of the copy number variation for GSTM1 and GSTT1 in patients with ischemic heart disease and ischemic cerebrovascular disease, showing no significant association with disease risk, even among smokers.

It has been well established that GSTP1 exhibits both antioxidant and glutathionylation activity, having important role in the maintenance of the cellular redox state [29]. Namely, GSTP1 is necessary for the activation of peroxiredoxin VI (Prdx6), a member of the family of antioxidant enzymes, which catalyzes detoxification of lipid peroxides, particularly in biological membranes [30]. After the exposure of endothelial cells to laminar shear stress, as a result of increase in free radical production, the upregulation of these antioxidant enzymes has been observed, probably as adaptive phenomenon [31]. Even more, their important role in regulating endothelial cell activation during atherosclerosis has been proposed. The most recent data on MCF-7 cells showed that the polymorphic expression of GSTP1 differentially interposes the Prdx6 activity, implying that depending upon their GSTP1 genotype, individuals will have significant differences in mounting an antioxidant response [30]. In carriers of the GSTP1 variant genotype, changed GSTP1 catalytic activity could deepen the progression of the disease, which consequently results in multiple cellular responses, such as DNA synthesis, transcription factor activation, and alteration of protein expression. If these results are translated to the HF setting, it may be speculated that GSTP1*Ile/Ile carriers might possibly have a higher antioxidant potential providing the favorable environment for better prognosis. Moreover, it is important to note that the highest HF risk was found for carriers of combined GSTA1*Val/GSTP1*Val variant alleles. Namely, GSTA1-1 is one of the most promiscuous GST enzymes with wide substrate specificity, including powerful antioxidant activity [32]. Thus, the presence of the GSTA1*Val gene variant, which results in lower expression of the enzyme, in combination with the GSTP1*Ile allele, might significantly contribute to decreased antioxidant capacity of HF patients, carriers of the combined GSTA1*Val/GSTP1*Val genotype. Regarding our previous data on the prognostic significance of oxidative stress and inflammatory parameters in HF [3, 7], we investigated whether GSTA1 and GSTP1 polymorphic variants could affect the plasma concentration of MDA, TNFα, and hs-CRP in our cohort of CAD-related HF patients. Indeed, we showed that carriers of the variant GSTP1*Val (rs1138272) genotype demonstrated higher TNFα levels, revealing new functional relevance of this GSTP1 polymorphism.

Aside from generation of reactive oxygen species (ROS) in the failing myocardium, endothelial activation also significantly contributes to myocyte apoptosis, necrosis, and

| Genotype                  | LVEDD* | LVESD* | FMD-NO* | FMD-NTG* |
|---------------------------|--------|--------|---------|----------|
| GSTP1*Ile105Val (rs1695)  |        |        |         |          |
| *Ile/Ile                  | 6.0 ± 0.8 | 4.8 ± 1.0 | 3.8 ± 4.1 | 12.4 ± 7.1 |
| *Ile/Val+*Val/Val         | 6.0 ± 0.7 | 4.6 ± 0.9 | 4.9 ± 5.1 | 11.3 ± 6.4 |
| GSTP1*Ala114Val (rs1138272) |       |        |         |          |
| *Ala/Ala                  | 6.0 ± 0.8 | 4.7 ± 0.9 | 4.4 ± 4.7 | 11.83 ± 7.1 |
| *Ala/Val+*Val/Val         | 6.0 ± 0.8 | 4.6 ± 1.1 | 5.51 ± 5.48 | 10.71 ± 5.1 |
| Combined GSTA1/GSTP1(rs1695) |      |        |         |          |
| *AA/*IleIle               | 6.3 ± 0.7 | 5.1 ± 0.8 | 3.8 ± 4.0 | 11.3 ± 7.4 |
| *AA/*IleVal+*ValVal       | 6.0 ± 0.7 | 4.6 ± 1.0 | 5.6 ± 4.8 | 11.4 ± 8.4 |
| *AB++BB/*IleIle           | 5.8 ± 0.9 | 4.5 ± 1.1 | 3.8 ± 4.2 | 13.1 ± 7.0 |
| *AB+BB/*IleVal+*ValVal    | 5.9 ± 0.7 | 4.5 ± 0.9* | 4.7 ± 5.3 | 11.2 ± 5.4 |
| Combined GSTA1/GSTP1(rs1138272) |      |        |         |          |
| *AA/*AlaAla               | 6.1 ± 0.6 | 4.8 ± 0.8 | 5.2 ± 4.9 | 11.6 ± 8.6 |
| *AA/*AlaVal+*ValVal       | 6.2 ± 0.9 | 4.9 ± 1.2 | 3.6 ± 2.5 | 10.7 ± 5.4 |
| *AB++BB/*AlaAla           | 5.9 ± 0.8 | 4.5 ± 0.9 | 3.9 ± 4.6 | 11.9 ± 6.2 |
| *AB+BB/*AlaVal+*ValVal    | 5.9 ± 1.0 | 4.5 ± 1.0 | 7.4 ± 7.3 | 9.5 ± 5.4 |

### 4. Discussion

Table 7: Echocardiographic and endothelial parameters stratified according to HF risk-associated GST genotypes in CAD subgroup.
remodeling of the extracellular matrix in the heart [33]. GSTP1 participates in regulation of stress signaling and apoptosis via its noncatalytic activity. Specifically, through protein:protein interaction, GSTP1 acts as an endogenous inhibitor of several signaling molecules, including c-Jun N-terminal kinase (JNK) [34] and TNF receptor-associated factor 2 (TRA2F) [35]. This interaction of GSTP1 with the MAPK and NF-xB axes of regulation is also responsible for its suggested anti-inflammatory role [36]. What is more, the degree of interaction between GSTP1 and JNK, a member of the mitogen-activated protein kinase (MAPK) signaling pathway, depends on the redox status of the cell. In that way, GSTP1 provides an important link between cellular redox potential and the regulation of kinase pathways involved in apoptosis and inflammation. The results of Andrukhova et al., showing elevated GSTP1 expression in the failing myocardium, were associated with reduced GSTP1/JNK interaction and consequent activation of the JNK-MAPK signaling cascade, essential for cardiomyocyte apoptosis [37], representing further confirmation of the contributing role of oxidative stress in the HF progression. Interestingly, the same authors indicated that a single dose of recombinant GSTP1 has cardioprotective effect in rats after myocardial infarction, affecting both inflammatory and apoptotic responses [37]. The substitutions of amino acid isoleucine (Ile) with valine (Val) at position 105 and alanine (Ala) with (Val) at position 114 as a consequence of GSTP1 polymorphisms can also affect the aforementioned interaction with JNK, causing an alteration in the GSTP1-mediated inhibitory effect of JNK activity [38]. Indeed, it has been shown that the GSTP1*C (Val105/Val114) haplotype is a more potent JNK inhibitor than the referent GSTP1*A (Ile105/Ala114) [38]. Although we did not find significant association between different GSTP1 haplotypes and the CAD-related HF risk, the obvious trend for decreasing HF risk in carriers of the GSTP1*C (Val105/Val114) haplotype might have a molecular explanation in its ability to prevent apoptosis more efficiently. A further indication of functional GST redundancy is provided by the fact that GSTA1 and GSTM1 were also capable of associating with JNK. Based on our results on increased disease risk for HF patient carriers of the combined GSTA1/GSTP1 variant genotype, it might be speculated that the impaired GSTP1/JNK interaction in CAD [37] could be further modified in patient carriers of the combined GSTA1/GSTP1 risk-associated genotype.

Finally, this seemingly “pleiotropic” modulatory role of GSTP1 was recently demonstrated for the key regulatory molecule of cell-cell communication in the heart, the signal transducer and activator of transcription 3 (STAT3) [39], STAT3 is fundamental for physiological homeostasis and stress-induced remodeling of the heart, as reviewed in the article of Haghikia et al. [40]. Furthermore, Chen et al. demonstrated that GSTP1, due to its interaction with STAT3, can inhibit angiotensin II-induced STAT3 activation of vascular smooth muscle cells (VSMCs) in vitro [39], thus preventing VSMC proliferation. However, the potential effect of GSTP1 polymorphism on this interaction still remains elusive.

Based on recent findings on increased GSTP1 catalytic activity in the metabolism of polycyclic aromatic hydrocarbons from tobacco smoke in carriers of the variant GSTP1*Val allele, which is located in the substrate-binding site, it may be hypothesized that GSTP1 genotyping could provide additional information not exclusively regarding tobacco smoking but also to other recognized GSTP1 substrates present in air pollutants, dietary compounds, products of endogenous metabolism, and lipid peroxidation. It would be tempting to investigate whether metabolism of ubiquitous reactive aldehyde acrolein, as a typical example of such a GSTP1 substrate associated with increased cardiovascular disease risk, would be affected by GSTP1 polymorphism [41]. In conclusion, due to involvement of GSTs in detoxification of xenobiots from tobacco smoke and also the metabolism of environmental and occupational pollutants, as well as various dietary constituents, different lifestyles might affect the role of GSTs within one population, while at the same time, the association observed in one may not be seen in other populations living in different environments.

To take further insight into molecular mechanisms and potential consequences of GSTP1 and GSTA1 polymorphisms with respect to CAD-related HF, we correlated the GSTP1 and GSTA1 variant genotypes with both the indices of heart remodeling and parameters of endothelial dysfunction. However, we found that only carriers of combined variant GSTA1*B/GSTP1*Val alleles had significantly decreased LVESD compared to individuals with GSTA1*AA/GSTP1*Ilele genotypes. Besides, the GSTP1 variant genotype was significantly associated with soluble ICAM-1 levels in these patients. As such, GSTP1 polymorphic variants may determine individual susceptibility to oxidative stress, inflammation, and endothelial dysfunction in HF, as well.

In our cohort of IDC patients, despite the evident trend, significant association of GSTP1 polymorphism with disease risk was not confirmed. The most probable reason is the small number of patients with IDC, having in mind the findings of Inoue et al. and Cannon et al. on the presence of coronary microcirculatory dysfunction despite angiographically normal epicardial coronary arteries in these patients [42, 43]. Hence, altered coronary flow reserve (CFR), which reflects coronary microvascular function and integrity, has been reported as an independent predictor of subsequent cardiac events in patients with idiopathic LV dysfunction [44].

Certain limitations could be considered in our study. The study findings may be influenced by potential biases arising from relatively small number of participants and GST polymorphisms studied. The number of patients was further reduced by analyzing subgroups of HF due to CAD and IDC. Furthermore, the use of population controls may have been more appropriate. Additionally, we cannot entirely rule out the possibility that some of our results could be caused by confounding, although we adjusted all results by age, gender, smoking, hypertension, and diabetes as potential confounding factors. Moreover, despite a large number of smokers in our cohort of HF patients, the correction for continuous measure of smoking was not determined.
5. Conclusions

It may be concluded that the variant GSTP1*Val allele is significantly associated with HF risk regardless of the specific cause. This association is even more potentiated in carriers of both GSTP1*Val and GSTA1*B alleles.

Data Availability

The database used to support the findings of this study is available from the corresponding author upon request.

Disclosure

The funding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of the data; in the writing of the manuscript; and in the decision to publish the results.

Conflicts of Interest

The authors declare no conflict of interest.

Acknowledgments

This work was supported by Grant 175052 from the Serbian Ministry of Education, Science and Technological Development.

References

[1] E. Braunwald, “Biomarkers in heart failure,” New England Journal of Medicine, vol. 358, no. 20, pp. 2148–2159, 2008.
[2] S. Radovanovic, A. Savic-Radojevic, M. Pljesa-Ercegovac et al., “Markers of oxidative damage and antioxidant enzyme activities as predictors of morbidity and mortality in patients with chronic heart failure,” Journal of Cardiac Failure, vol. 18, no. 6, pp. 493–501, 2012.
[3] A. Savic-Radojevic, S. Radovanovic, T. Pekmezovic et al., “The role of serum VCAM-1 and TNF-α as predictors of mortality and morbidity in patients with chronic heart failure,” Journal of Clinical Laboratory Analysis, vol. 27, no. 2, pp. 105–112, 2013.
[4] A. Savic-Radojevic, M. Pljesa-Ercegovac, M. Matic, D. Simic, S. Radovanovic, and T. Simic, “Novel Biomarkers of Heart Failure,” Advances in Clinical Chemistry, vol. 79, pp. 93–152, 2017.
[5] K. F. Ayoub, N. V. K. Pothineni, J. Rutland, Z. Ding, and J. L. Mehta, “Immunity, inflammation, and oxidative stress in heart failure: emerging molecular targets,” Cardiovascular Drugs and Therapy, vol. 31, no. 5–6, pp. 593–608, 2017.
[6] A. M. Rababa’ah, A. N. Guillore, R. Mustafa, and T. Hijjawi, “Oxidative stress and cardiac remodeling: an updated edge,” Current Cardiology Reviews, vol. 14, no. 1, pp. 53–59, 2018.
[7] S. Radovanovic, M. Krotin, D. V. Simic et al., “Markers of oxidative damage in chronic heart failure: role in disease progression,” Redox Report, vol. 13, no. 3, pp. 109–116, 2008.
[8] S. Radovanovic, A. Savic-Radojevic, T. Pekmezovic et al., “Uric acid and gamma-glutamyl transferase activity are associated with left ventricular remodeling indices in patients with chronic heart failure,” Revista Española de Cardiología, vol. 67, no. 8, pp. 632–642, 2014.
[9] A. Bhatnagar, “Environmental cardiology: studying mechanistic links between pollution and heart disease,” Circulation Research, vol. 99, no. 7, pp. 692–705, 2006.
[10] D. J. Conklin and A. Bhatnagar, “Are glutathione S-transferase null genotypes “null and void” of risk for ischemic vascular disease?,” Circulation: Cardiovascular Genetics, vol. 4, no. 4, pp. 339–341, 2011.
[11] F. M. F. Alameddine and A. M. Zafari, “Genetic polymorphisms and oxidative stress in heart failure,” Congestive Heart Failure, vol. 8, no. 3, pp. 157–164, 172, 2007.
[12] C. J. Henderson and C. R. Wolf, “Disruption of the glutathione transferase pi class genes,” Methods in Enzymology, vol. 401, pp. 116–135, 2005.
[13] T. Simic, A. Savic-Radojevic, M. Pljesa-Ercegovac, M. Matic, and J. Mimic-Oka, “Glutathione S-transferases in kidney and urinary bladder tumors,” Nature Reviews Urology, vol. 6, no. 5, pp. 281–289, 2009.
[14] H.-L. Yeh, L.-T. Kuo, F.-C. Sung, C.-W. Chiang, and C.-C. Yeh, “GSTM1, GSTT1, GSTP1, and GSTA1 genetic variants are not associated with coronary artery disease in Taiwan,” Gene, vol. 523, no. 1, pp. 64–69, 2013.
[15] V. Kovacs, B. Gasz, B. Balatonyi et al., “Polymorphisms in glutathione S-transferase are risk factors for perioperative acute myocardial infarction after cardiac surgery: a preliminary study,” Mol. Cell. Biochem., vol. 389, no. 1–2, pp. 79–84, 2014.
[16] R. Polimanti, S. Piacentini, N. Lazzarin, M. A. Re, D. Manfioletto, and M. Fucirelli, “Glutathione S-transferase variants as risk factor for essential hypertension in Italian patients,” Molecular and Cellular Biochemistry, vol. 357, no. 1–2, pp. 227–233, 2011.
[17] C. Marinho, I. Alho, D. Arduino, L. M. Falcão, J. Brás-Nogueira, and M. Bicho, “GST M1/T1 and MTHFR polymorphisms as risk factors for hypertension,” Biochemical and Biophysical Research Communications, vol. 353, no. 2, pp. 344–350, 2007.
[18] J. D. Hayes, J. U. Flanagan, and I. R. Jowsey, “Glutathione transferases,” Annual Review of Pharmacology and Toxicology, vol. 45, no. 1, pp. 51–88, 2005.
[19] D. Zhou, W. Hu, Q. Wang, and Y. Jin, “Glutathione S-transferase M1 polymorphism and coronary heart disease susceptibility: a meta-analysis involving 47,596 subjects,” Heart, Lung and Circulation, vol. 23, no. 6, pp. 578–585, 2014.
[20] J.-J. Tang, M.-W. Wang, E. Jia et al., “The common variant in the GSTM1 and GSTT1 genes is related to markers of oxidative stress and inflammation in patients with coronary artery disease: a case-only study,” Molecular Biology Reports, vol. 37, no. 1, pp. 405–410, 2010.
[21] A. Phulukdaree, S. Khan, D. Moodley, and A. A. Chuturgoon, “GST polymorphisms and early-onset coronary artery disease in young South African Indians,” South African Medical Journal, vol. 102, no. 7, pp. 627–630, 2012.
[22] J. Ping, H. Wang, M. Huang, and Z.-S. Liu, “Genetic analysis of glutathione S-transferase A1 polymorphism in the Chinese population and the influence of genotype on enzymatic properties,” Toxicological Sciences, vol. 89, no. 2, pp. 438–443, 2006.
[23] L. W. Harries, M. J. Stubbins, D. Forman, G. C. Howard, and C. R. Wolf, “Identification of genetic polymorphisms at the glutathione S-transferase pi locus and association with...
susceptibility to bladder, testicular and prostate cancer,” Carci-
nogenesis, vol. 18, no. 4, pp. 641–644, 1997.

[24] S. Z. Abdel-Rahman, R. A. el-Zein, W. A. Anwar, and W. W. Au, “A multiplex PCR procedure for polymorphic analysis of GSTM1 and GSTT1 genes in population studies,” Cancer Letters, vol. 107, no. 2, pp. 229–233, 1996.

[25] M. Matic, T. Pekmezovic, T. Djukic et al., “GSTA1, GSTM1, GSTP1, and GSTT1 polymorphisms and susceptibility to smoking-related bladder cancer: a case-control study,” Urologic Oncology: Seminars and Original Investigations, vol. 31, no. 7, pp. 1184–1192, 2013.

[26] M. S. Norskov, P. Carmeliet, V. Adler, Z. Yin, S. Y. Fuchs et al., “Dynamic limitation of coronary vasodilator reserve in patients with dilated cardiomyopathy and chest pain,” Journal of the American College of Cardiology, vol. 10, no. 6, pp. 1190–1200, 1987.

[27] M. V. Raynolds, M. R. Bristow, E. W. Bush et al., “Angioten-
sin-converting enzyme DD genotype in patients with ischemic or idiopathic dilated cardiomyopathy,” The Lancet, vol. 342, no. 8879, pp. 1073–1075, 1993.

[28] J. E. Sanderson, R. P. Young, C. M. Yu, S. Chan, J. A. J. H. Critchley, and K. S. Woo, “Lack of association between insertion/deletion polymorphism of the angiotensin-converting enzyme gene and end-stage heart failure due to ischemic or idiopathic dilated cardiomyopathy in the chinese,” The American Journal of Cardiology, vol. 77, no. 11, pp. 1008–1010, 1996.

[29] K. D. Tew, Y. Manevich, C. Grek, Y. Xiong, J. Yys, and D. M. Townsend, “The role of glutathione S-transferase P in signaling pathways and S-glutathionylation in cancer,” Free Radical Biology and Medicine, vol. 51, no. 2, pp. 299–313, 2011.

[30] Y. Manevich, S. Hutchens, K. D. Tew, and D. M. Townsend, “Allelic variants of glutathione S-transferase P1-1 differentially mediate the peroxidase function of peroxiredoxin VI and alter membrane lipid peroxidation,” Free Radical Biology and Medicine, vol. 54, pp. 62–70, 2013.

[31] A. L. Mowbray, D.-H. Kang, S. W. Kang, and H. Jo, “Laminar shear stress up-regulates peroxiredoxins (PRX) in endothelial cells: PRX 1 as a mechanosensitive antioxidant,” Journal of Biological Chemistry, vol. 283, no. 3, pp. 1622–1627, 2008.

[32] B. F. Coles and F. F. Kadlubar, “Human alpha class glutathione S-transferases: genetic polymorphism, expression, and suscep-
tibility to disease,” Methods in Enzymology, vol. 401, pp. 9–42, 2005.

[33] P. Carmeliet, “Mechanisms of angiogenesis and arteriogen-
esis,” Nature Medicine, vol. 6, no. 4, pp. 389–395, 2000.

[34] V. Adler, Z. Yin, S. Y. Fuchs et al., “Regulation of JNK signaling by GSTP,” The EMBO Journal, vol. 18, no. 5, pp. 1321–1334, 1999.

[35] Y. Wu, Y. Fan, B. Xue et al., “Human glutathione S-transferase P1-1 interacts with TRAF2 and regulates TRAF2-ASK1 sig-
als,” Oncogene, vol. 25, no. 42, pp. 5787–5800, 2006.

[36] B. Xue, Y. Wu, Z. Yin et al., “Regulation of lipopolysaccharide-
duced inflammatory response by glutathione S-transferase P1 in RAW264.7 cells,” FEBS Letters, vol. 579, no. 19, pp. 4081–4087, 2005.

[37] O. Andrukhova, M. Salama, M. Krssak et al., “Single-dose GSTP1 prevents infarction-induced heart failure,” Journal of Cardiac Failure, vol. 20, no. 2, pp. 135–145, 2014.

[38] A. F. Thévenin, C. L. Zony, B. J. Bahnson, and R. F. Colman, “GST pi modulates JNK activity through a direct interaction with JNK substrate, ATF2,” Protein Science, vol. 20, no. 5, pp. 834–848, 2011.