The role of protein Z and protein Z-dependent protease inhibitor polymorphisms in the development of prosthetic heart valve thrombosis

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Introduction

Protein Z (PZ) is a vitamin K-dependent factor, which is mainly synthesized by the liver. It acts as an activator of a serpin, namely, protein Z-dependent inhibitor (ZPI), which inhibits factor Xa. In human plasma, ZPI is present in more quantity than PZ, and PZ and PZI are present as a complex in circulation . Individuals have a wide range of normal plasma PZ concentrations, which may be partly explained by genetics (1, 2). The A-13G polymorphism in the promoter of the PZ gene, G-103A in intron A, or G-79A in intron F is associated with decreased plasma PZ level. The potential role of alterations in PZ and/or ZPI levels in the pathogenesis of thrombotic and/or hemorrhagic diseases has been previously investigated in several studies (3, 4).

Prosthetic valve thrombosis (PVT) is a severe complication of cardiac valve replacement. In several circumstances, it may be difficult to identify the precipitating factors of PVT despite therapeutic international normalized ratio (INR). Therefore, it is necessary to discover new factors that may be responsible for the development of PVT. We aimed to evaluate the role of PZ/ZPI polymorphisms in the development of PVT. To the best of our knowledge, this is the first study to demonstrate this.

Ten consecutive patients with PVT and 10 consecutive healthy prosthetic valve patients without a history of thrombosis, miscarriage, venous thromboembolism, transient ischemic attack, and cerebrovascular accident were enrolled in this prospective, observational, and case-control study. All study subjects were Turkish people who had been residing in Turkey for at least one generation. Written informed consent was obtained from the participants. Patients with infective endocarditis, moderate-to-severe paravalvular regurgitation, pannus growth over mechanical valves, active infection, a history of systemic inflammatory process, malignancy, end-stage liver disease, renal failure, and other hematologic diseases were excluded from the study.

Transthoracic echocardiography, two-dimensional transesophageal echocardiography (TEE), and real-time three-dimensional TEE were performed in all patients. Thrombus was recognized as a homogeneous mobile or fixed mass with an echodensity similar to that of the myocardium located at the valve occluder and/or valve struts and was visualized in all patients by echocardiography (5–7).

Blood samples were obtained from all participants in EDTA-containing tubes. We extracted gDNA from approximately 5x106 leukocytes using the QIAamp DNA Mini Kit (QIAGEN) according to the manufacturer's recommendations. PCR products were purified by adding 8 µL PCR product to the mixture containing 0.5 µL exonuclease I (Thermo) and 1 µL rAPid Alkaline Phosphatase (Roche) at 37°C for 70 min and 72°C for 20 min. For mutational analysis, minisequencing was performed by adding 1 µL SnaPshot Multiplex mixture (Applied BioSystem, Forster City, USA) and 5 pmol minisequencing-specific primer to 1 µL purified PCR products, which were then subjected to 25 cycles at 96°C for 10 s, 50°C for 5 s, and 60°C for 30 s. The products of minisequencing were analyzed using an ABI PRISM 3100 Avant Genetic Analyser (Applied Biosystems, Forster City, USA).

The demographics and clinical characteristics of the study subjects and controls are given in Table 1. In the PVT group, non-obstructive thrombosis (NOT) was detected in five patients and obstructive thrombosis (OT) was detected in the remaining five patients. Seven (70%) patients with PVT had a previous history of thrombolytic therapy (TT), and four (40%) had suffered prior thromboembolic event.

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Table 1. Clinical characteristic of patients and controls

Characteristics	PVT (n=10)	Control (n=10)
Age, years	53±18	48±10
Gender, male	4	5
Admission INR	1.8±0.4	2.4±0.5
TESS, months	95±50	103±76
Hypertension, n	2	3
DM, n	1	1
Dyslipidemia, n	1	1
AF, n	3	3
Localization of prosthesis		
Aortic, n	3	4
Mitral, n	5	5
Aortic + Mitral, n	2	1

AF - atrial fibrillation; DM - diabetes mellitus; INR - international normalized ratio; PVT - prosthetic valve thrombosis; TESS - time elapsed since surgery

The frequencies of PZ and ZPI polymorphisms are included in Table 2. PZ polymorphism was detected in seven PVT patients (one patient had PZ-G-79A and six patients had A-13G) and three control subjects (one patient had PZ-G-79A and two patients had A-13G). Heterozygotic R67X was observed in two patients with PVT, whereas it was not detected in any of the control subjects. Furthermore, W303X polymorphism was not detected in any patient in both groups.

Of the five patients with OT, one had heterozygotic G-79A, one had homozygotic A-13G, two had heterozygotic A-13G PZ polymorphism, and one had a normal variant. Of the five patients with NOT, one had heterozygotic R67X mutation, one had both heterozygotic R67X and heterozygotic A-13G mutation, one had homozygotic A-13G mutation, and one had only heterozygotic A-13G mutation; the remaining one patient did not have any PZ/ZPI polymorphism.

In the past three decades, PZ and ZPI system and their contribution to the pathological cascade have been studied with great interest. PZ acts as a cofactor for the inactivation of activated factor X (FXa) by protein ZPI. ZPI, a member of the serpin superfamily of proteinase inhibitors, inhibits FXa in a protein Z-dependent, Ca^{2+}-dependent, and phospholipid-dependent fashion and inhibits FXIa in the absence of cofactors (8, 9). A meta-analytical study demonstrated that low levels of PZ could be an essential risk factor for all thrombotic events such as arterial thrombosis, pregnancy complications, and venous thromboembolic diseases (4). ZPI was also associated with cardiovascular events and thrombosis (10). The present study is the first to investigate the potential additional role of PZ/ZPI polymorphisms in the pathogenesis of PVT.

This study included a small number of subjects; therefore, statistical analysis could not be performed, making it a descriptive study. However, the above results demonstrate that PZ/ZPI polymorphisms may play a role in the development of PVT.

Table 2. Comparison of PZ/ZPI polymorphisms between PVT and control groups

		PVT (n=10)	Control (n=10)
PZ G-79A			
Alleles	A	1 (5%)[a]	1 (5%)
	G	19 (95%)	19 (95%)
Genotypes	Wild type (GG)	9 (90%)	9 (90%)
	Heterozygote (GA)	1 (10%)	1 (10%)
	Homozygote (AA)	0	0
PZ A-13G			
Alleles	A	12 (60%)	17 (85%)
	G	8 (40%)	3 (15%)
Genotypes	Wild type (AA)	4 (40%)	8 (80%)
	Heterozygote (AG)	4 (40%)	1 (10%)
	Homozygote (GG)	2 (20%)	1 (10%)
ZPI R67X			
Alleles	C	18 (90%)	20 (100%)
	T	2 (10%)	0
Genotypes	CC	8 (80%)	10 (100%)
	CT	2 (20%)	0
	TT	0	0
ZPI W303X			
Alleles	A	0	0
	G	20 (100%)	20 (100%)
Genotypes	Wild type (GG)	10 (100%)	10 (100%)
	Heterozygote (GA)	0	0
	Homozygote (AA)	0	0

a - allele and genotype frequencies (percentage); A - adenine; C - cytosine; G - guanine; PZ - protein Z; PVT - prosthetic valve thrombosis; T - thymine; ZPI - protein Z-dependent inhibitor

Conclusion

ZPI polymorphisms may play a role in the development of PVT. However, large, independent, prospective, population-based, and more comprehensive studies with different ethnicities are required to evaluate the relationship between PZ/ZPI polymorphisms and PVT.

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