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Letter to the Editors-in-Chief

Decreased incidence of EPCR 4678G/C SNP in multiple myeloma patients with thrombosis

Dear Editors,

Patients with Multiple Myeloma (MM) have an increased risk of thrombosis [1]. The underlying pathogenetic mechanism has not been fully elucidated. Several disease-specific factors have been implicated in the pathogenesis of hypercoagulation. Apart from non specific factors (age, immobility, comorbidities), qualitative and quantitative defects in coagulation and fibrinolytic factors, acquired protein C resistance, as well as high proinflammatory cytokine levels are considered as triggering factors [1]. It is also well established that endothelial perturbing therapies, such us chemotherapy and immunomodulatory agents (thalidomide, lenalidomide), enhance the risk of thrombosis in patients with MM [1,2]. Since classic inherited thrombophilia (FVLeiden 1691G/A, FII 20210G/A) has not been definitely proved to influence the risk of thrombosis in MM patients [2,3], we hypothesized that single nucleotide polymorphisms (SNPs), which are related with perturbed endothelium, might contribute to increased susceptibility to thrombosis in these patients.

The endothelial protein C receptor (EPCR) exerts a key role in the anticoagulation pathway of protein C (PC) [4]. Protein C circulates in plasma in an inactive form. Its conversion to active protein is computed on the cellular surface of endothelial cells. Endothelial protein C receptor binds circulating PC and "presents" it at the thrombin- thrombomodulin complex. Thrombin, which is activated by thrombomodulin, converts PC in activated protein C (APC). Endothelial protein C receptor enhances the rate of PC activation by 5 to 20 fold [5]. Activated protein C together with its cofactor protein S (PS) inactivates the procoagulant factors Va and VIIIa, by proteolytic cleavage and via this mechanism the PC pathway regulates fibrin formation. Endothelial protein C receptor is primarily expressed on large vessel endothelium, in placental vessels and at traces in most capillary beds [4–6]. It also contributes to the anti-inflammatory, anti-apoptotic and cytoprotective function of APC, which is thought to be mediated by protease-activated receptor-1 (PAR-1) signaling. Additionally, there are suggestions that due to its structural similarity to the major histocompatibility class I family of proteins, EPCR may be directly involved in the immune response. Recently, the possible role of EPCR as a multi ligand receptor has been examined with evidence that FVII and FVIIa and even FXa can bind to EPCR on the unperturbed endothelium in vivo [7].

Several polymorphisms in the EPCR gene have been reported and some of them have been associated with the risk of thrombosis [8]. Among them, the single nucleotide polymorphism (SNP) **4600A/G**, found in exon 4, results in a Serine to Glycine substitution at residue 219 in the transmembrane domain and is associated with increased levels of soluble endothelial protein C receptor (sEPCR) and increased thrombotic risk. On the other hand, the **4678G/C** SNP, found in the 3'Untranslated Region (3'UTR), is associated with high levels of circulating APC and reduced risk of thrombosis. However, there is contradictory data in the literature regarding the influence of these polymorphisms in the risk of thrombosis [5,6,8].

We performed a retrospective study in order to evaluate the implication, if any, of the above mentioned SNPs of the EPCR gene in the risk of thrombosis in MM patients. We studied 11 patients with MM, 5 males and 6 females with a mean age of 67,9 years (range from 40 to 77 years) who developed thrombosis. Six patients were receiving lenalidomide, 2 patients were receiving thalidomide based regimens and 2 were undergoing Autologous Stem Cell Transplantation (ASCT). Five patients were also receiving Erytropoietin (EPO). All patients were under thromboprophylaxis according to the current Guidelines for prevention and management of thrombosis in Multiple Myeloma [2,9]. More specifically 9 patients were receiving aspirin. There were 4 patients that have undergone 2 thrombotic events. During the first episode they were on aspirin prophylaxis, while the second event occurred although they were on prophylaxis with acenocumarol (2 patients), low molecular weight heparin (LMWH) (1patient) and aspirin (1 patient) respectively. The mean time from the first to the second thrombotic episode was three months. One patient was receiving acenocumarol due to previous venous thromboembolism (VTE) (prior to the diagnosis of MM). Three patients underwent pulmonary embolism (PE). Thrombosis was objectively confirmed (Ultrasonografy, Color Doppler, Computed Tomography). The median time to VTE since the initiation of treatment was 2 months (range from 1 to 24 months). The study was approved by the Medical Ethical Committee on human research of Alexandra General Hospital (University of Athens, School of Medicine). Genomic DNA was isolated from peripheral blood leucocytes. The samples were tested for the 4600A/G and 4678G/C EPCR SNPs. They were also genotyped for the FVLeiden 1691A/G polymorphism and Prothrombin 20210A/G mutation with the method of polymerase chain reaction (PCR) and reverse hybridization (StripAssay®, ViennaLab Austria). The frequencies of EPCR, FVLeiden, FII20210G/A alleles in MM patients were compared with the prevalence of the above mentioned SNPs in a Greek adult healthy population [10]. Allele frequencies were calculated by gene counting. The z-test for proportions was used to compare the percentages of alleles within patients and controls. A two tailed p value < 0.05 was considered as statistically significant. Patients characteristics along with genotyping for FVLeiden, FII20210G/A, EPCR 4600G/A and EPCR 4678G/C are depicted in Table 1.

The frequency of EPCR 4600A and 4600G allele in MM patients was 0.909 and 0.091 respectively. There was no difference when compared to controls. However there was a statistically significantly lower frequency of the 4678C allele of the EPCR gene in MM patients, compared to controls [0.228 Vs 0.460 ($p = 0.034$)]. One patient was found to be heterozygous for FVLeiden and carried one 4678C allele (4678GC genotype) and 2 patients were found to be heterozygous for the prothrombin 20210G/A mutation one with 4678GC genotype and one with 4678GG genotype. According to the literature 4678C allele has a protective effect on the risk of thrombosis [5,6]. EPCR 4678C allele is located on the 3'UTR and this region has been reported to possess functional

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Table 1
Patients characteristics.

PATIENT	GENDER	AGE* (YEARS)	MM TYPE	THERAPEUTIC TREATMENT*	THROMBOPROFYLAXIS	THROMBOSIS	FVLEIDEN	FII 20210A/G	EPCR 4600A/G	EPCR 4678C/G
1	M	73	IgGκ	L + EPO	ASPIRIN	24 MONTHS (DVT)	NO	NO	AA	GG
2	M	70	IgGλ	L	ASPIRIN	24 MONTHS (DVT + PE)	NO	NO	AG	GG
3	F	76	IgGκ	VMP + EPO	ASPIRIN	14 MONTHS (DVT)	NO	HET	AA	GC
				L	SINTROM	2 MONTHS (DVT)				
4	M	70	FLC λ	L + EPO	ASPIRIN	10 MONTHS(DVT)	NO	NO	AA	GC
5	F	77	IgGκ	L + GCSF	ASPIRIN	17 MONTHS (PE)	NO	NO	AG	GC
				L + EPO	SINTROM	4 MONTHS (DVT)				
6	F	68	IgGκ	L	ASPIRIN	1 MONTH	NO	NO	AA	GG
7	F	71	IgGκ	VAD/T/D	ASPIRIN	2 MONTHS (DVT + PE)	NO	NO	AA	GG
				VAD CAELYX	LMWH	PE				
8	M	64	FLC κ	ASCT/EPO	SINTROM (DUE TO PREVIOUS DVT)	<1 MONTHS (DVT)	NO	NO	AA	GC
9	M	61	IgGλ	TD	ASPIRIN	2 MONTHS (DVT)	HET	NO	AA	GC
				TVD	ASPIRIN	<1 MONTH (DVT)				
10	F	49	IgGκ	V/D/ASCT	ASPIRIN	<1 MONTH (DVT)	NO	NO	AA	GC
11	F	N/A	N/A	N/A	N/A	N/A	NO	HET	AA	GG

L = Lenalidomide, V = Velcade, M = Melphalan, P = Prednisone, D = Dexamethasone, T = Thalidomide, DVT = Deep Vein Thrombosis, PE = Pulmonary Embolism, GCSF = Granulocyte Colony Stimulating Factor, ASCT = Autologous Stem Cell Transplantation, EPO = Erythropoietin, NO = Normal, HET = Heterozygous.
*AGE at the time of thrombosis. *Therapeutic treatment at the time of thrombosis.

transcription binding sites. Carriers of one 4678C allele (4678GC genotype) have higher APC levels than non carriers (4678GG genotype) while homozygous for 4678C allele (4678CC genotype) have even higher APC levels. EPCR 4678C allele has been described to have a protective effect also in carriers of FVLeiden [6]. Thus, carriership of the C allele leads to higher levels of APC and the pathophysiological mechanism seems to be the enhanced gene transcription, synthesis of the EPCR protein and expression of the EPCR on the membrane surface [6]. The reduced frequency of the protective 4678C allele and the subsequent abolishment of its protective action in MM could contribute to the already known disease hypercoagulability and to the existing risk of thrombosis. This finding should be confirmed by additional clinical studies with larger series of patients. We should also highlight that from the 4 patients that have experienced 2 thrombotic episodes, one had undergone surgery and radiation therapy, one had no change in his thromboprophylactic regimen after the first thrombotic episode and two of them, although were carriers of one 4678C EPCR allele, were also heterozygous for FVLeiden and for FII20210G/A respectively. We could hypothesize that in these patients classic thrombophilia may have aggravated their risk of thrombosis.

This is the first report in the literature that could suggest a putative role of EPCR SNPs in the development of thrombosis in MM. Although with the implementation of international guidelines and anticoagulant interventions we expect that thromboembolic events will even decrease in the future, we could propose that analysis for SNPs of EPCR gene (an endothelium perturbation indication) could facilitate stratification of MM patients in terms of risk of thrombosis and thus guide proper anticoagulation, especially in high risk patients.

Conflict of Interest Statement

The authors state that they have no conflict of interest.

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