Thrombophilia in thalassemia major patients: analysis of genetic predisposing factors

Thromboembolic phenomenona have been described in patients with thalassemia major. In a multicenter, retrospective study we investigated the effect of factor V (FV Leiden), prothrombin (FII), methylene-tetrahydrofolate reductase (MTHFR), PIA2 glycoprotein IIIa (GpIIIa) and factor VII gene polymorphisms on the risk of thrombosis in thalassemic patients. A higher prevalence of the FVII H7 allele in subjects without thrombosis suggests that this genetic marker confers protection against thrombosis.

Thromboembolic events, such as recurrent and transient ischemic cerebral attacks, strokes, pulmonary embolism, deep venous thrombosis, and portal vein thrombosis, have been observed in thalassemia major patients[1] with a prevalence ranging from 2.5% to 4%.[2] The hypercoagulable state has been attributed to a wide variety of hemostatic alterations including platelet hyperaggregability, protein C and antithrombin deficiency, increased urinary excretion of thromboxane A2 metabolites, enhanced expression of P-selectin, and procoagulant alterations of red cells.[3,4] Few studies have evaluated the impact of prothrombotic polymorphisms in thalassemia thrombotic patients.[3,5] The aim of our study was to determine the frequency of selected prothrombotic polymorphisms (FV, FII, MTHFR, GpIIIa, and FVII) in a group of thrombotic thalassemic patients and to assess whether the co-inheritance had an impact on the thrombotic manifestations.

We made a retrospective analysis of clinical history and hospital records of all cases of thrombosis spanning 10 years (1991-2000) by means of a questionnaire sent to the Italian centers for thalassemia care. Thirty-three patients (18 men and 15 women) with thalassemia major and a history of one or more episodes of thrombosis were identified from a total of 580 thalassemic patients. The age range of these 33 patients was 6-59 years, mean 26.88±10.03. Twenty-nine were splenectomized. Their mean pre-transfusional Hb was 9.07±0.3 g/dL and mean transfusional interval 22±3 days. Data on the same number of thalassemic subjects without thrombosis (20 men and 13 women; aged between 7-52 years, mean 26.73±9.58; 20/33 splenectomized, pre-transfusional Hb 9.01±0.4 g/dL and mean transfusional interval 23±4 days) were also collected. All patients were treated with subcutaneous desferrioxamine infusion at doses established according to the severity of iron overload.

DNA analysis was performed by polymerase chain reaction amplification, followed by digestion with restriction enzymes and electrophoresis on polyacrylamide gel. FV Leiden, FII, MTHFR, and GpIIIa gene mutations were determined as previously described.[6-9] The hypervariable region 4 of intron 7 of the FVII gene was amplified according to a modification of the method described by Marchetti *et al.*[10] Three alleles were identified: a common allele (H6) of 443 bp with six monomers, a less frequent allele (H7) of 480 bp with seven monomers of 37 bp, and a very rare allele (H5) of 406 bp with five monomers.

Allele and genotype frequencies of the five polymorphisms in thrombotic and non-thrombotic thalassemic patients were compared by chi-squared or Fisher's exact tests. The relative risk of thrombosis in thalassemic carriers of the H7 allele of the FVII gene versus thalassemic non-carriers and the 95% confidence interval were estimated by logistic regression analysis using SPPS 10.0 for Windows software.

From the retrospective multicenter analysis, the estimated prevalence of thromboembolic events was 5.6% (33/580). Throm-

Table 1. Genotypes and allele frequencies in thalassemic patients with thrombosis (G1), thalassemic patients without thrombosis (G2) and healthy subjects (G3).

	C677T MTHFR		*G1691A FV*		*G20210A F II*		*C1565T GpIIb/IIIa*		*4 HPVR F VII*	
	Genotypes (n=33) n (%)	*Alleles (chr=66) n (%)*	*Genotypes (n=33) n (%)*	*Alleles (chr=66) n (%)*	*Genotypes (n=33) n (%)*	*Alleles (chr=66) n (%)*	*Genotypes (n=33) n (%)*	*Alleles (chr=66) n (%)*	*Genotypes (n=33) n (%)*	*Alleles (chr=66) n (%)*
G1 (n=33)	CC 7(21)	WT 32(52)	GG 0	WT 63(95)	GG 26(79)	WT 59(89)	CC 26(79)	WT 59(89)	H5/6-H6/6 27(82)	H5 0
	CT 18(55)		GA3(9)		GA7(21)		CT 7(21)		H6/7 6(18)	H6 60(91)
	TT8(24)	Mut 34(48)	AA 30(91)	MUT 3(5)	AA1(3)	MUT 7(11)	TT 0	Mut 7(11)	H7/7 8(24)	H7 6(9)
G2 (n=33)	CC 5(15)	WT 39(59)	GG 32(97)	WT 65(99)	GG 32(97)	WT 61(92)	CC 21(64)	WT 53(80)	H5/6-H6/6 14(42)	H5 1(1)
	CT 17(52)		GA1(3)		GA1(3)		CT 11(33)		H6/7 13(39)	H6 40(61)
	TT 11(33)	Mut27(41)	AA0	MUT 1(1)	AA 0	MUT 5(8)	TT 1(3)	Mut13(20)	H7/7 5(15)	H7 25(38)
G3 (n=33)	CC 9(24)	WT 30(45)	GG 32(97)	WT 65(99)	GG 32(97)	WT 63(95)	CC 29(88)	WT 62(94)	H5/6-H6/6 24(72)	H5 0
	CT 20(61)		GA1(3)		GA1(3)		CT4(12)		H6/7 7(21)	H6 55(83)
	TT5(15)	Mut 36(55)	AA0	MUT 1(1)	AA0	MUT 3(5)	TT0	Mut 4(6)	H7/7 8(24)	H7 11(17)

boembolic events consisted of portal vein thrombosis (n=9), pulmonary embolism (n=4), deep vein thrombosis (n=5), superficial thrombophlebitis (n=6), intra-atrial thrombosis (n=2), retinal thrombosis (n=1), CNS thrombosis (n=2), deep vein thrombosis associated with pulmonary embolism (n=2), thrombophlebitis and pulmonary embolism (n=1), and ictus plus intra-atrial thrombosis (n=1).

Table 1 shows the genotype and allele frequencies in thalassemic patients with thrombosis (group 1) and thalassemic patients without thrombosis (group 2). There were no significant differences between the thrombotic thalassemic group and the control group for all analyzed polymorphisms with the exception of the FVII polymorphism. The H7 allele was significantly less frequent among case patients (9%) than in non-thrombotic patients (38%) ($p = 0.00008$ by Fisher's exact test, combining the H5 allele with the H6 alleles). Accordingly, only six thrombotic patients were heterozygous for the H7 allele (18%) compared with 13 heterozygotes and six homozygotes in the non-thrombotic group ($p = 0.001$ by Fisher's exact test, combining the H7 heterozygotes and homozygotes in a single class). This resulted in a relative risk of thrombosis of 0.164 (95% CI 0.053-0.503) in thalassemic carriers of the H7 allele versus non carriers. There were no significant differences between the two groups when we analyzed the prevalence of combined risk factors (data not shown). In conclusion, the key finding of our study deals with the risk of thrombotic events associated with polymorphism in the FVII gene. The significantly higher prevalence of the H7 allele in hypervariable region 4 of intron 7 in subjects without thrombosis suggests that this genetic marker could confer a relative protection against thrombosis. A similar observation on the frequency of H7 alleles was noted in familiar myocardial infarction.[11]

On the other hand, other factors, common in thalassemic patients, such as chronic hepatitis, cirrhosis, and iron overload may facilitate a hypercoagulable state.[12]

Achille Iolascon,[a,m] Paola Giordano,[a] Sonia Storelli,[a] Hui-Hua Li,[b] Brigida Coppola,[a] Antonio Piga,[c] Elisabetta Fantola,[d] Gianluca Forni,[e] Paolo Cianciulli,[f] Silverio Perrotta,[g] Carmelo Magnano,[h] Aurelio Maggio,[i] Antonio Mangiagli,[l] Marcella Devoto[b]

[a]Dipartimento di Biomedicina dell'Età Evolutiva Università degli Studi di Bari; [b]Genetic Epidemiology Research Lab. Al duPont Hospital for Children, Wilmington DE; [c]Dipartimento di Scienze Pediatriche e dell'Adolescenza Università di Torino; [d]Dipartimento di Medicina Interna, Istituti Clinici di Perfezionamento, Milano; [e]Centro della Microcitemia e delle Anemie Congenite, Ospedale Galliera, Genova; [f]Ospedale S. Eugenio, Day Hospital Talassemici, Roma; [g]Dipartimento di Pediatria II Università di Napoli; [h]Servizio di Thalassemia P.O. Garibaldi, Catania; [i]Divisione di Ematologia II con Talassemia, Palermo; [l]Centro Microcitemie A.O. Umberto 1°, Siracusa; [m]Clinica Pediatrica Università di Foggia

*Correspondence: Prof. Achille Iolascon, Istituto di Pediatria, Università degli Studi di Foggia, viale L. Pinto, Foggia, Italy.
Phone: international +39.080.5478923
E-mail: a.iolascon@bioetaev.uniba.it*

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