

Supplementary Material to de la Morena-Barrio et al. “Regulatory regions of *SERPINC1* gene: Identification of the first mutation associated with antithrombin deficiency” (Thromb Haemost 2012; 107.3)

Suppl. Table 1: Oligonucleotides used for PCR amplification of the *SERPINC1* gene. E:

Exon; I: Intron. F: forward primer; R: reverse primer.

Amplicon	Primer name	Oligonucleotide sequence (5´-3´)	Size (bp)
5´ region	AT_-1F	GGACCTTATTAACATCTGAC	743
	AT_-728R	CCAAGTTCACAGGGCGCATG	
	AT_-660F	GGAGTCCTTGATCACACAGCA	1088
	AT_IN1R	GTCTTTGACTGTAACCTACCAG	
	AT_0,5F	GGCTACTGTTCACACTGCACATG	1067
	AT_1R	GAAAGCTCACCCCTCTTAC	
E1-E2	AT_1F	CTGTCCTCTGGAACCTCTGCGAGA	2880
	AT_2R	GGTTGAGGAATCATTGGACTTG	
I1	AT_19F	ATCCGGGAAGAGAGCAAATGC	1200
	AT_31R	CTCAAACAGCAACAACAAAC	
E2-E3	AT-2F	TGCAGCCTAGCTTAACTTGGCATT	3180
	AT_3aR	AGAGGAAGAACTCGGAGGTCAGG	
E3-E4	AT_3aF	AACTAGGCAGCCCACCAAACCC	1410
	AT_3bR	GAAGAGCAAGAGGAAGTCCCT	
E4-E5	AT_3bF	TTGAATAGCACAGGTGAGTAGGTT	1380
	AT_4R	AAGGGAGGAAACTCCTTCCTAG	
E5	AT_4F	TGTGTTCTTACTTTGTGATTCTCT	391
	AT_4R	AAGGGAGGAAACTCCTTCCTAG	
E5-E6	AT_4F	TGTGTTCTTACTTTGTGATTCTCT	2521
	AT_5R	CATGCATCTCCTTTCTGTACCC	
I5-E6	AT_95F	TGGAGAGGAATTTGAAAG	1800
	AT_5R	CATGCATCTCCTTTCTGTACCC	
I5	AT_95F	TGGAGAGGAATTTGAAAG	1080
	AT_106R	AACATCTTTCTTTCCAGTCTG	
E6-E7	AT_5F	TTCTCCCATCTCACAAAGAC	3660
	AT_6R	AGAGGTGCAAAGAATAAGAA	
I6	AT_130F	GGGAGACTAGGGTGTGTA	1200
	AT_142R	CAGATCTAGAGGGAAACACC	
I6-E7	AT_130F	GGGAGACTAGGGTGTGTA	1860
	AT_6R	AGAGGTGCAAAGAATAAGAA	
3´ region	AT_130F	GGGAGACTAGGGTGTGTA	2860
	AT_159R	ACACACAAGTAATAACATCCAC	
	AT_3´F	TTATCTTCATGGGCAGAGTAGC	1000
	AT_159R	ACACACAAGTAATAACATCCAC	

Suppl. Table 2: Genotyping by PCR-allele specific restriction assay and High Resolution

Melting. Mutated nucleotides are underlined.

Polymorphism	Amplification/Probe Oligonucleotides	PCR Size (bp)	Method of genotyping
rs2227588	AT_0.5F: GGCTACTGTTCA <u>C</u> ACTGCACATG AT_-728R: CCAAGTTCACAGGGC <u>G</u> CATG	198	Mnl I (NEB)
rs2227590	AT_18F: CATTTC AAGT GCTCTCCCTCC AT_19R: GCATTGCTCTCTTCC <u>C</u> GAT	203	FokI (Takara)
rs2227596	AT_30F: ATCTTCAGCACACAGATATTG AT_33R: GCTGGGTGTGGTGGCGC	229	Tsp 45I (NEB)
rs2227603	AT_53F: TTGAGACCAGGGGCCAG AT_57R: AATTGACCACTAGAATAGAAG	408	FokI (Takara)
rs941989	AT_60F: GCTTCCAAACCACACATGTTC AT_62R: CCATGCTCTCTCCTCAGGC	241	HinfI (Promega)
rs2227607	AT_71F: GACTGCTGGAGGGCTGAC AT_73R: GAACTGGCATCTTCAAAACACCC	186	NlaIII (NEB)
rs2759328	AT_112F: TCCAAACTGAATTCCCATCTG <u>C</u> GG AT_5R: CATGCATCTCCTTTCTGTACCC	190	FauI (Fermentas)
rs677	AT_114F: TGAGTACACCTTCCCCAC AT_115R: CTGTATTATAGCAGGCCTGTGG	126	DdeI (NEB)
rs2227616	AT_130F: GGGAGACTAGGGTGTGTA AT_133R: CCGGGCACGGTGGCTCATGCC <u>G</u> TAA	332	BstEII (NEB)
rs5778785	AT_95F: TGGAGAGGAATTTGAAAG AT_97R: GTGAGCCGAGATCGCACCGC	178	HRM
g.2085 T>C (rs78972925)	AT_13F: GAGGGAGTGTGGGCAAGAGA AT_13R: AGGGTTAAGCAAAGTGTAGAG Razor: ACCCGGACTAACTTGAAT	107 Razor probe	HRM/Razor probe

NEB: New England BioLabs; HRM: High Resolution Melting.

Suppl. Table 3: Polymorphisms identified in healthy blood donors with low (L1: 73.5%; L2: 74.5% and L3: 75.1%) and high (H1: 114.4% and H2: 116.1%) antithrombin levels.

ins: insertion; I: Intron; E: exon; WT: Wild-Type allele; ND: not determined; S: small nucleotide series; L: large nucleotide series.

Polymorphism	Change	Location	Position	H1	H2	L1	L2	L3
g.2085 T>C (rs78972925)	T>C	Promoter	-228	T/C	T/T	T/T	T/T	T/T
rs3138521	S>L	Promoter	-376	S/L	S/S	S/L	ND	S/S
rs2227589	G>A	I 1	182	G/G	G/G	G/A	G/G	G/G
rs2227590	C>T	I 1	361	C/C	C/C	C/C	T/C	C/C
rs2227592	G>A	I 1	686	G/G	G/G	G/A	G/G	G/G
rs2227596	A>G	I 1	1605	A/G	A/A	A/A	ND	A/A
rs2227603	T>G	I 2	3850	T/G	T/T	T/T	ND	T/T
rs941988	G>A	I 2	4505	G/G	G/G	G/A	ND	G/G
rs941989	G>A	I 2	4527	G/A	G/G	G/A	ND	G/G
rs2227607	G>T	I 3	5646	G/T	G/G	G/G	ND	G/G
rs2227609	G>T	I 4	6836	G/T	G/G	G/G	G/G	G/G
rs5877	A>G	E 5	7536	A/G	A/A	A/G	A/G	A/A
rs5878	A>G	E 5	7566	A/G	A/A	A/G	A/G	A/A
rs1799876	T>C	I 5	7927	C/C	T/T	C/C	ND	T/T
rs5778785	<i>ins</i> TTATTA	I 5	8073	<i>ins/ins</i>	WT	WT	WT	WT
rs57195053	(ATT) _n *	I 5	9231	14/5	14/14	14/11	14/16	14/14
rs2759328	G>A	I 5	9693	G/G	G/G	A/A	G/G	G/G
rs677	G>C	I 6	9837	G/G	G/G	G/G	G/C	G/G
rs2227616	<i>delA</i>	I 6	11794	A/-	A/A	A/A	A/A	A/A

Numbering is based on genomic DNA sequence, nucleotide +1 corresponds to the A of the ATG initiation codon of the *Homo sapiens* antithrombin gene (GenBank accession number NG_012462.1).

* The WT allele contains (ATT)₁₄, while the reported allelic variant contains (ATT)₁₃ in the intron 5 Alu sequence.

Suppl. Table 4: Clinical and genetic features of patients with moderate antithrombin deficiency as the single thrombophilic defect identified.

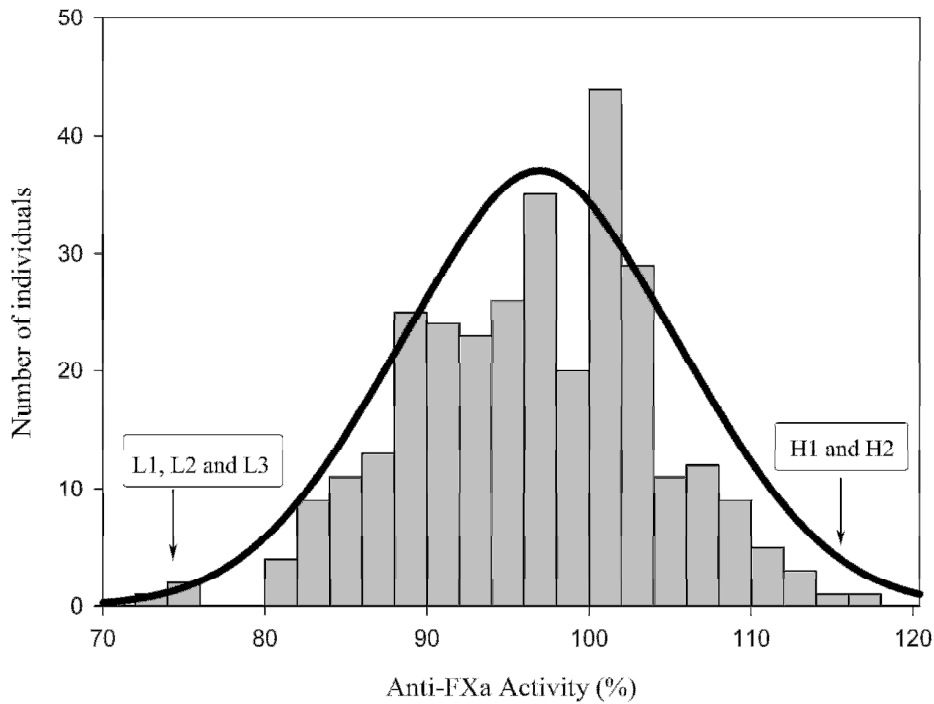
N	Sex	Thrombotic event	Age first event	Anti-FXa	Polymorphisms
P1	Male	DVT	47	72	rs2227588(A/G)* rs61827938 (A/G)
P2	Male	Recurrent DVT	66	77	No
P3	Female	DVT	28	66	No
P4	Male	Recurrent DVT + PE	66	86	No
P5	Male	DVT	59	79	No
P6	Male	DVT	35	70	No
P7	Male	Recurrent arterial thrombosis	48	78	rs3138521(L/L) rs2227588(A/G)* rs61827938 (A/G)
P8	Male	DVT	36	88	No
P9	Male	Recurrent DVT	65	86	No
P10	Male	Recurrent DVT	67	81	No
P11	Male	Recurrent DVT	68	79	No
P12	Male	DVT	68	78	No
P13	Male	Recurrent DVT + PE		72	No
P14	Male	Stroke	87	76	rs3138521(S/L)

DVT: Deep venous thrombosis. PE: Pulmonary embolism.

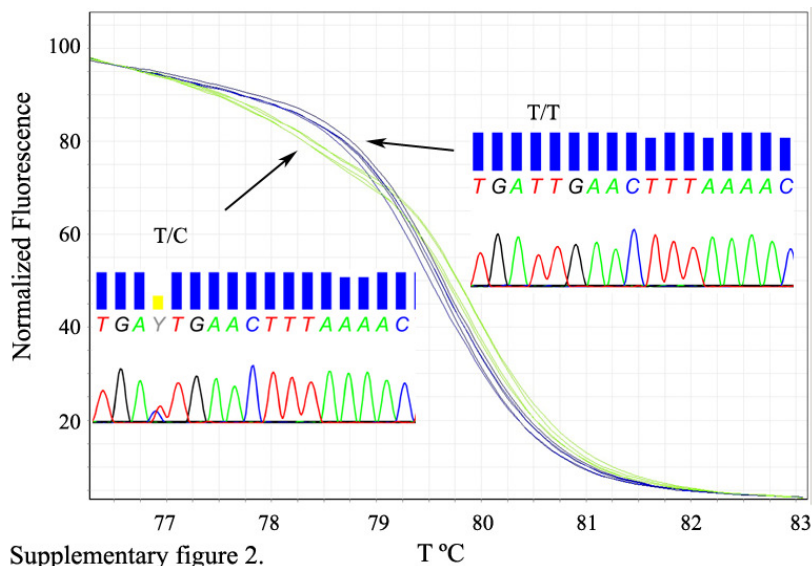
rs3138521: S: small nucleotide series; L: large nucleotide series.

* rs61827938 and rs2227588 are in complete disequilibrium linkage.

Supplementary figure 1.

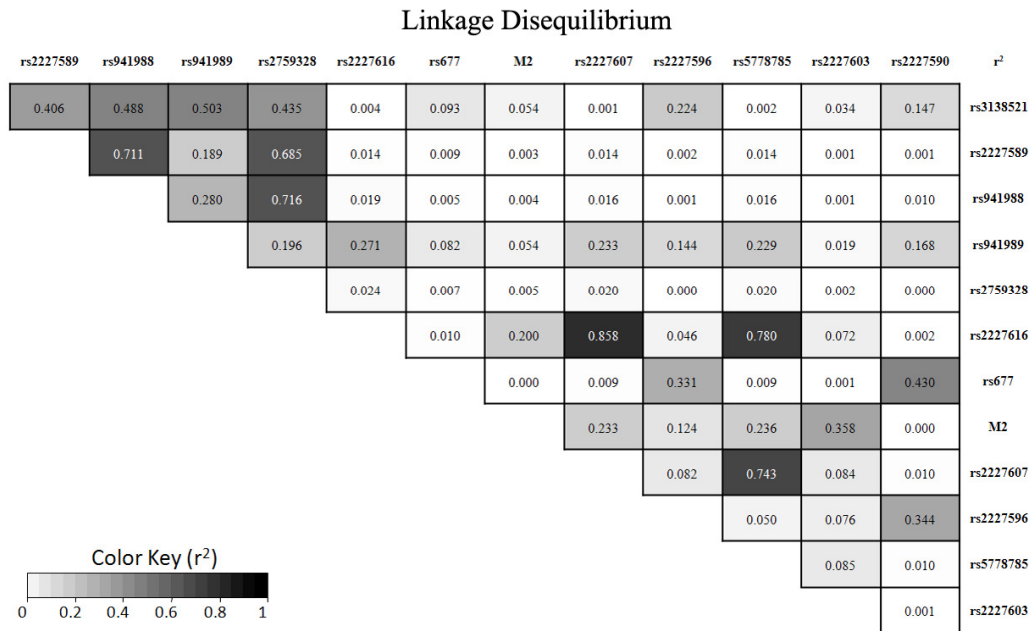


Suppl. Figure 1: Distribution of antithrombin anti-FXa activity in 307 Spanish blood donors. Subjects with low (L) or high (H) antithrombin levels selected for *SERPINC1* sequencing are also indicated.



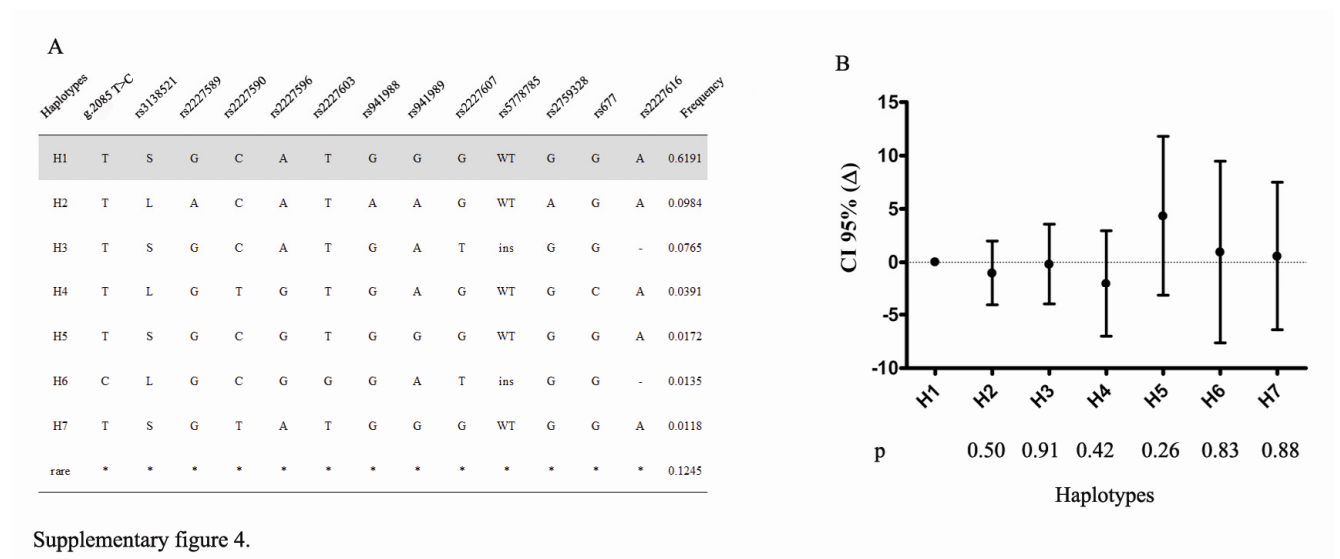
Supplementary figure 2.

Suppl. Figure 2: HRM-razor melting curves and electropherograms of wild type and heterozygous g.2085 T>C genotypes.



Supplementary figure 3.

Suppl. Figure 3: Linkage disequilibrium (r² values) between polymorphisms identified in our study. A grey scale is used to associate r² values.



Supplementary figure 4.

Suppl. Figure 4: Haplotype analysis. A) Haplotypes identified. WT: Wild-type allele; ins: ATTATT insertion. B) Correlation between the haplotypes and anti-FXa activity.