Table 1. Missense and nonsense mutations in the *F8* gene identified in Portuguese families with haemophilia A.

Exon	Family/Patient Identification	Nucleotide Change	Type of Mutation / Recurrency	Codon Change	FVIII:C (%)	FVIII:Ag (%)	Severity	[i]Inhibitors	Comments	[†]Conservation	[g]References
Exon 2	[c]F1503/0271	c.206T>C	Non-CpG transition	L50P	<1	4.2	Severe	Neg.	Familial; steric clashes, misfolding and secretion/stability defect	LVVVV-	Present study
	[c]F1503/0417	c.206T>C		L50P	<1	3.4	Severe	Neg.			Present study
Exon 3	F524	c.292G>A	Non-CpG transition	E79K	2.4	3.1	Moderate	Neg.	*De novo*; maternal origin; disrupts a salt bridge with residue R3	EEEEE-	(21)
Exon 4	F277	c.491G>A	Non-CpG transition	G145D	1	ND	Severe	Neg.	*De novo*; coil residue buried in A1 domain and secretion/stability defect	GGGGG-	(21)
	F370	c.515G>T	Transversion	C153F*	1.8	1.9	Moderate	Neg.	Familial; disrupts the disulphide bridge with C179 and secretion/stability defect	CCCCC-	Present study
	F4181	c.545A>C	Transversion	D163A*	4	ND	Moderate	Neg.	Familial; loss of A1/A3 interdomain H-bonds	DDDDD-	Present study
	F245	c.553A>G	Non-CpG transition	K166E*	3	ND	Moderate	Neg.	Familial; core residue close to the A1/A3 interface with steric clashes	KKKKR-	Present study
.	F3291	c.556G>T	Transversion	D167Y	<1	1.6	Moderate	Neg.	Sporadic; loss of H-bonds with steric clashes and secretion/stability defect	DDDDD-	Present study
Exon 7	F2845	c.899A>C	Transversion	H281P*	1	ND	Severe	Neg.	Familial; disrupts a H-bond with S524 and probably decreased FVIIIa stability	HHHHH-	Present study
	[b]F215	c.902G>A	CpG transition/ Recurrent	R282H	7	ND	Moderate	Neg.	Familial; decreased FVIIIa stability	RRRRR-	(22)
	[b]F1202	c.902G>A	Recurrent	R282H	4	ND	Moderate	Neg.	Familial		
	[b]F2531	c.902G>A	Recurrent	[h]R282H	5	80	Moderate	Neg.	Familial		
Exon 8	F80 Lisboa2	c.1043G>C	Transversion	C329S	2.6	3.2	Severe to moderate	Neg.	Familial; intracellular retention and increased rate of A2 subunit dissociation	CCCCC-	(10,18)
	F401	c.1063C>T	CpG transition	R336X	2	ND	Severe to moderate	Neg.	Sporadic; premature termination of translation	RRRQQ-	Present study
Exon 9	F2970	c.1349A>G	Non-CpG transition	Y431C*	10	ND	Mild	Neg.	Sporadic; disrupts a H-bond with R439 and probably illegitimate disulphide bonding	YYYYY-	Present study
	F35	c.1349A>G	Non-CpG transition	Y431C*	9.7	8	Mild	Neg.	Familial	YYYYY-	Present study
Exon 10	[a]F140	c.1492G>A	CpG transition	G479R	13.4	22	Mild	Neg.	Sporadic; core residue, steric clash with R531	GGGGG-	(6,14)
	[a]F177 Porto1	c.1492G>A		G479R	15.8	31.6	Mild	Neg.	Familial		
	[a]F1573	c.1492G>A		G479R	10.0	NA	Mild	Pos, Type 1	Familial		
Exon 11	[a]F805	c.1589A>G	Non-CpG transition	Y511C*	11.8	9	Moderate	Neg.	Familial; steric clashes and probably illegitimate disulphide with secretion defect	YYYYY-	Present study
	[a]F1090	c.1589A>G		Y511C*	10	10	Mild	Neg.	Familial		Present study
	[a]F1224	c.1589A>G		Y511C*	9	8	Mild	Neg.	Familial		Present study
	[a]F2825	c.1589A>G		Y511C*	5	ND	Mild	Neg.	Sporadic		Present study
	[a]F2864	c.1589A>G		Y511C*	15	11	Mild	Neg.	Familial		Present study
	[a]F3775	c.1589A>G		Y511C*	13	7	Mild	Neg.	Sporadic		Present study
	[a]F3844	c.1589A>G		Y511C*	15	ND	Mild	Neg.	Familial		Present study
	[a]F3905	c.1589A>G		Y511C*	17.5	9	Mild	Neg.	Familial		Present study
	[a]F803	c.1636C>T	CpG transition	R527W	[e]12	NA	Mild	Neg.	Familial; defect in interaction with FIXa	RRRRR-	(24)
	[a]F2043	c.1636C>T		R527W	[e]NA	NA	NA	NA	Sporadic; obligate carrier tested		
	[b]F346	c.1648C>T	CpG transition	R531C	15	21	Mild	Neg.	Sporadic; increased rate of A2 subunit dissociation	RRRRR-	(24,25)
	[a]F486	c.1648C>T		R531C	[e]9.5	11	Mild	NA	Sporadic		

	[a]F2324	c.1648C>T		R531C	[e]32	13	Mild	Neg.	Sporadic		
	[b]F2972	c.1648C>T	Recurrent	R531C	[e]32	ND	Mild	Neg.	Familial		
	F1565	c.1649G>A	CpG transition	R531H	[e]33	84	Mild	Neg.	Familial; increased rate of A2 subunit dissociation		(24,25)
	F1093	c.1702G>A	Non-CpG transition	G549S*	1	2.7	Severe	Neg.	Familial; steric clashes with severe misfolding and lack of secretion	GGGGG-	Present study
Exon 12	F1669	c.1804C>T	CpG transition	R583X	<1	<1	Severe	Neg.	Sporadic; maternal somatic mosaicism; premature termination of translation	RQRQR-	Present study
	F463	c.1834C>T	CpG transition	R593C	12.5	ND	Mild	NA	Sporadic, intracellular accumulation	RRRRR-	(26)
Exon 13	F2558	c.1933C>T	CpG transition	Q626X	<1	NA	Severe	Pos, Type 2	Familial; premature termination of translation	QQQEE-	Present study
	F184	c.2044G>T	Transversion	V663F	7	NA	Mild	NA	Familial; carrier tested;	VVVVV-	Present study
Exon 14	F4240	c.2150G>A	CpG transition	R698Q*	44	63	Mild	Neg.	Familial; mild functional defect and enhanced A2 domain dissociation	RRRRRR	Present study (27)
	F816	c.2167G>A	CpG transition	A704T	12.5	ND	Mild	Neg.	Familial; impaired interaction with FIXa	AAAAAA	Present study
	F1853	c.3031A>T	Transversion	K992X*	<1	<1	Severe	Neg.	Sporadic; premature termination of translation	KKKKSL	Present study
	[b]F199	c.5122C>T	CpG transition	R1689C	4.3	60%	Moderate	Neg.	Familial; thrombin activation site	RRRRRR	Present study
	[b]F400	c.5122C>T	Recurrent	R1689C	5	ND	Moderate	Neg.	Familial; thrombin activation site		Present study
	[b]F2624	c.5122C>T	Recurrent	R1689C	9	115	Moderate	Neg.	Fporadic; thrombin activation site		Present study
	F2399	c.5144G>C	Transversion	R1696P*	3	NA	Moderate	Neg.	Familial; obligate carrier tested; abolish a H-bond with D1769, misfolding and secretion/stability defect	RRRRRR	Present study
	F1378	c.5159C>A	Transversion	A1701D*	<1	1	Severe	Neg.	Familial; steric clashes with severe misfolding and secretion/stability defect	AAAAAA	Present study
	F3162	c.5219G>T	Transversion	R1721M*	2	1.2	Severe	Neg.	Sporadic; probably affects splicing, FXa cleavage site	RRRRRT	Present study
Exon 15	[a]F569	c.5275G>A	Non-CpG transition	D1740N*	3	30	Moderate	Neg.	Sporadic; new glycosylation site with interference in its interaction with FIXa	DDDDDD	Present study
	[a]F2843	c.5275G>A	Non-CpG transition	D1740N*	5	20	Moderate	Neg.	Sporadic;	DDDDDD	Present study
	F2739	c.5321A>G	Non-CpG transition	[I]H1755R*	<2	1	Severe	Neg.	Familial; severe steric clashes with misfolding and secretion/stability defect	HHHHHH	Present study
	F1203	c.5363A>G	Non-CpG transition	D1769G*	5	8.2	Moderate	Neg.	Familial; abolish a H-bond with R1696, misfolding and secretion/stability defect	DDDDDD	Present study
Exon 18	F436	c.58788C>T	CpG transition	R1941X	<1	ND	Severe	Pos, Type 1	Familial; premature termination of translation	RRRRRR	Present study
	F194Porto2	c.5900G>A	CpG transition	G1948D	7.4	48.7	Moderate to mild	Neg.	Sporadic; intracellular retention and increased rate of A2 subunit dissociation	GGGGGG	(10)
	F70	c.5953C>T	CpG transition	R1966X	<1	<1	Severe	Pos, Type 1	Familial; premature termination of translation	RRRRRR	(11)
	F1390	c.5954G>A	CpG transition	[h]R1966Q	14	35	Mild	Neg.	Familial; disrupt a salt bridge with residue D1828 and of a H-bond with K661	RRRRRR	(21)
Exon 21	F185	c.6243G>C	Transversion	W2062C*	<1	<1	Severe	Pos, Type 2	Familial; probably illegitimate disulphide bonding with secretion/stability defect	WWWWW-	Present study
Exon 23	F2611	c. 6497C>T	CpG transition	R2147X	1.2	1	Severe	Neg.	Sporadic; premature termination of translation	RRQRR-	Present study
	F1656	c. 6506G>A	CpG transition	R2150H	5	1	Mild	Neg.	Sporadic; implicated in vWF interaction	RRRRR-	(29)
Exon 24	[a]F430	c.6222C>G	Transversion	Q2189E	23.5	ND	Mild	Neg.	Sporadic; disrupt a H-bond and presumably mild secretion/stability defect	QQQQQ-	Present study
	[a]F2676	c.6222C>G	Transversion	Q2189E	27.6	29	Mild	Neg.	Sporadic		Present study
	[a]F3605	c.6222C>G	Transversion	Q2189E	28	ND	Mild	Neg.	Sporadic		Present study
	[a]F3606	c.6222C>G	Transversion	Q2189E	20	ND	Mild	Neg.	Sporadic		Present study
	[a]F4136	c.6222C>G	Transversion	Q2189E	23	ND	Mild	Neg.	Sporadic		Present study
	F3848	c.6682C>T	CpG transition	[J]R2209X	<1	ND	Severe	Pos. transitory	Sporadic; premature termination of translation	RRRRR-	Present study

Exon 25	F739	c.6793C>A	Transversion	Q2246K*	10	4	Mild	Neg.	Familial; refolding associated with moderate secretion/stability defect	QQQQQ–	Present study
Exon 26	[c]F181/0736	c.6910G>A	Non-CpG transition	G2285R*	2.4	1	Moderate	Neg.	Familial; severe steric clashes with misfolding and secretion/stability defect	GGGGG–	Present study
	[c]F181/0150	c.6910G>A		G2285R*	3	ND	Moderate	Neg.	Familial		
	[c]F181/2068	c.6910G>A		G2285R*	3	ND	Moderate	Neg .	Familial		
	[a]F574	c.6910G>A		G2285R*	2.4	1	Moderate	Neg.	Familial		
	[d]F211	c.6910G>A		G2285R*	7	3.7	Moderate to mild	Neg.	Familial		
	[d]F258	c.6910G>A		G2285R*	3.5	5.5	Moderate to mild	Neg.	Familial		
	[d]F284	c.6910G>A		G2285R*	4.3	2.5	Moderate to mild	Neg.	Familial		
	[a]F2495	c.6910G>A		G2285R*	4.2	2.8	Moderate	Neg.	Familial		

Nucleotide +1 corresponds to position 172, i.e., the A of the first Met codon, of the sequence with GeneBank Accession No. NM_000132. The nomenclature system for the mutations is according to den Dunnen and Antonarakis (20). Recurrency: i.e. independent occurrence in the Portuguese population established by haplotype analysis. [†]Single letter amino-acid code of the index residue in FVIII of six species: *Homo sapiens*, *Sus scrofa*, *Canis familiaris*, *Mus musculus*, *Rattus norvegicus, Gallus gallus* (partial sequence). Novel mutations are indicated by *. NA- not available. ND- not done. [a]Related by haplotype; [b]Unrelated by haplotype; [c]Patients from the same family; [d]Belong to the same kindred. [e]Mutation with one stage/two-stage activity discrepancy. [f]Type 1, high responder and Type 2 low responder. [g]Reference regarding the comments. [h]Mutation segregating with the polymorphic FVIII-1241E variant. [i]Mutation segregating with the polymorphic FVIII-2238V variant. [j]Mutation segregating with the silent polymorphism c.2118A>G at codon L687 and with the polymorphic FVIII1310C variant. [k]Found in association with the recently reported vWF polymorphism c.2771G>A, R924Q (37).

Table 2. Insertions and deletions in the *F8* gene identified in Portuguese families with haemophilia A.

Exon	Patient Identification	Nucleotide Change	[c]DNA sequence	Codon Change	FVIII:C (%)	FVIII:Ag (%)	Severity	Inhibitors	Comments	References
Exon 2	F916	c.205_206delCT	ACT **CT**G TTT	L50fsX12*	<1	<1	Severe	Neg.	Sporadic; African origin	Present study
Exon 3	F196	c.305_306insCTGAGATTTATGTTCTTA	GAT AC^A TGT	V84fsX	1	ND	Severe	Neg.	Sporadic	Present study
Exon 13	F1206	c.2100_2105delATGGA	TCG **ATG GA**A	M682fsX26*	<1	ND	Severe	Neg.	Familial	Present study
Exon 14	F4143	c.2593delG	GGG **G**AC ATG	D846fsX12*	<1	1.3	Severe	Pos. transitory	Sporadic	Present study
	F1205	c.2614_2615insGAGT	CCT GAG T^CA	S853fsX9*	<1	ND	Severe	Neg.	*De novo*; maternal origin	Present study
	[a]F300	c.2945_2946insA	GGA AAA A^AT	V964fsX7	5	ND	Moderate	Neg.	Familial	Present study
	[a]F674	c.2945_2946insA	GGA AAA A^AT	V964fsX7	<1	ND	Moderate	Neg.	Familial	Present study
	[a]F2550	c.2945_2946insA	GGA AAA A^AT	V964fsX7	<1	ND	Severe	Neg.	Familial	Present study
	F1822	from IVS13 -1341 to c.3291	[d]ctctcaga-CAGAAA	Large deletion*	1	1	Severe	Neg.	Sporadic; 4044bp deletion	Present study
	F876	c.3302_3303delAG	AAA G**AG** GGC	E1082fsX15*	<1	ND	Severe	Neg.	Familial; carrier tested	Present study
	[a1]F466	c.3637delA	GAA AAA AAA **A**TT	I1194fsX4	<1	NA	Severe	NA	Sporadic	Present study
	[a1]F1207	c.3637delA	GAA AAA AAA **A**TT	I1194fsX4	1.8	ND	Moderate	Neg.	*De novo*; maternal origin	Present study
	[a2]F2217	c.3637delA	GAA AAA AAA **A**TT	I1194fsX4	<1	ND	Severe	Pos. type	*De novo*; maternal origin; African origin	Present study
	[a2]F2614	c.3637delA	GAA AAA AAA **A**TT	I1194fsX4	1.6	1	Moderate	Neg.	Familial	Present study
	[b]F2719	c.3637delA	GAA AAA AAA **A**TT	I1194fsX4	1	ND	Severe	Neg.	Familial	Present study
	F1374	c.3870_3871insA	AA^A GGG GAG	G1272fsX28	<1	ND	Severe	Neg.	Familial	Present study
	F143	c.4293_4297delGAGAA	AA**C TCT T**CT	H1415fsX5*	<1	<1	Severe	Neg.	Familial.	Present study
	[b]F24 Porto3	c.4379_4380insA	AAA A^AT AAC	N1441fsX1	1	<1	Severe	Neg.	*De novo*; maternal grandfather	(6)
	[a]F61	c.4379_4380insA	AAA A^AT AAC	[c]N1441fsX1	<1	<1	Severe	Neg.	Familial	Present study
	[b]F379	c.4379_4380insA	AAA A^AT AAC	N1441fsX1	<1	NA	Severe	Neg.	*De novo*; maternal origin	Present study
	[a]F1376	c.4379_4380insA	AAA A^AT AAC	N1441fsX1	<1	1.2	Severe	Neg.	*De novo*; maternal origin	Present study
	F566	c.4482delG	ACT **G**TT CTC*	V1479fsX78*	1	ND	Severe	Neg.	Sporadic	Present study
	F1636	c.4691_4692insTTTC	CCC **TTT C**^TG	R1548fsX21*	<1	ND	Severe	Neg.	Sporadic	Present study
	F1398	c.4825_4826insA	GAA AAA ^ACA	T1590fsX3	<1	ND ?	Severe	Neg.	Sporadic	Present study
	F2844	c.5142_5144delACG	AACA **CG**A C	[f]R1696del*	1	12	Severe to moderate	Pos. type 1	Familial; in-frame deletion	Present study
Exon 15	F4302	c.5337delG	TG GG**G** CCA*	P1761fsX4*	<1	<1	Severe	Pos, Type 2	Sporadic	Present study
Exon 18	F2215	c.5965_5967delGAG	A **GAG** GAG **T**	E1970del*	40	46	Mild	Neg	Familial; in-frame deletion	Present study
Exon 24	F2448	c.6652_6653delAT	AAT **AT**G TTT	M2199fsX18*	<1	1	Severe	Neg	Sporadic	Present study

For mutations in tandem repeats the position of the last unchanged 3' nucleotide is indicated. Novel mutations are indicated by *. [a]Related by haplotype; [b]Unrelated by haplotype; [c]Deleted nucleotides are in bold; the position of insertions are indicated by ^; slipped nucleotides are underlined. [d]Intronic sequence is in lower case while exonic nucleotides are in upper case. [e]Combined FVII/FVIII deficiency. [f]Mutation segregating with the silent polymorphism c.3864A>C at codon S1269.

Table 3. Mutations in the splice site regions of the *F8* gene identified in Portuguese families with haemophilia A.

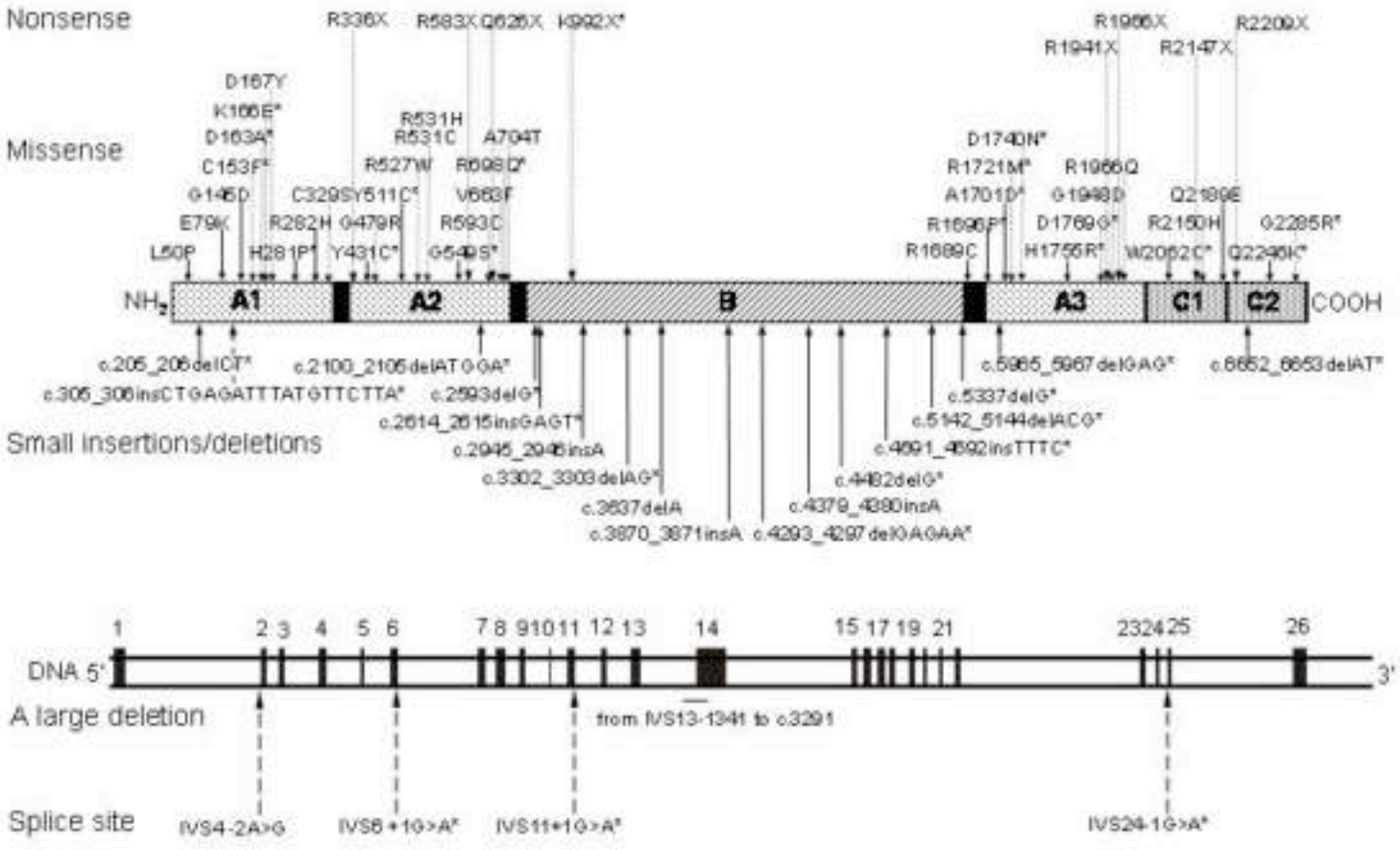
Intron	Patient Identification	Nucleotide Change	DNA sequence	CVN	CVM	FVIII:C (%)	FVIII:Ag (%)	Severity	Inhibitors	Comments	References
Intron 4	F62 Lisboa1	IVS4-2A>G	ttt**c**ag GG	0.955	0.716	1.7	1.3	Severe	Neg.	Familial; in-frame skipping of exon 5 and 4-5	(18)
Intron 6	F2551	IVS6+1G>A*	AG **g**tatgt	0.887	0.705	1	1	Moderate to mild	Neg.	NA; in-frame skipping of exons 4 to 7	Present study
Intron 11	F2533	IVS11+1G>A*	AG **g**tgagt	0.967	0.785	1	1	Severe	Pos. type 1	Sporadic; in-frame skipping of exons 10 and 11	Present study
Intron 24	F2672	IVS24-1G>A	cctca**g** GT	0.952	0.714	<1	<1	Severe	Neg.	Familial; in-frame skipping of exon 25 with or without exon 19	Present study; (33)

Novel mutations are indicated by *. Mutated nucleotide is in bold. CVN wild-type splice site consensus value; CVM mutated splice site consensus value.

Table 4. Portuguese haemophilia A families without an obvious causative *F8* mutation and screened for mutations in the FVIII-binding region of vWF.

	F8			*vWF*								
Patient Identification	Exon	Nucleotide Change	Codon Change	Exon	Nucleotide Change	Codon Change	FVIII:C (%)	FVIII:Ag (%)	vWF:Ag (%)	Severity	[a]Inhibitors	Comments
F1103	–	No identified *F8* mutation		–	No type 2N vWF mutation		9	ND	ND	Mild	Neg	Familial
F1377	14	c.3780C>G	[a]D1241E	–	No type 2N vWF mutation		1	ND	Severe	Familial	[b]Pos, Type 1	Familial
F3543	–	No identified *F8* mutation		–	No type 2N vWF mutation		2.5	ND	NDE	Moderate	Neg	Sporadic
[c]F1705	–	No identified *F8* mutation		19	[c.2446C>T]+[c.2446C>T]	R816W	4	6	70	Moderate	Neg	Familial
[d]F2832	19	[e]c.6003T>C	V1982V	19	[c.2446C>T]+[?]	R816W	ND	–	–	Mild	–	Sporadic

[a]Polymorphic FVIII-1241E variant. [b]Type 1, high responder. [c]Female patient. [d]Daughter of a haemophilia A patient with FVIII:C of 6%, not available for the study. [e]Apparently silent mutation c.6003T>C at codon V1982.


Nonsense
R336X
R583X
Q626X
K992X*
R1966X
R1941X
R2147X
R2209X
Missense
D167Y
K166E*
D163A*
C153F*
G146D
E79K
L50P
R282H
H281P*
C329S
Y431C*
R527W
R531H
R531C
G479R
Y511C*
R598Q*
A704T
V683F
G549S*
R593C
D1740N*
R1721M*
A1701D*
R1696P*
R1689C
R1966Q
G1948D
D1769G*
H1755R*
Q2199E
R2150H
W2052C*
R2285R*
Q2246K*
NH2
A1
A2
B
A3
C1
C2
COOH
Small insertions/deletions
c.205_206delCT*
c.305_306insCTGAGATTTATGTTCTTA*
c.2100_2105delATGGA*
c.2593delG*
c.2614_2615insGAGT*
c.2945_2946insA
c.3302_3303delAG*
c.3637delA
c.3870_3871insA
c.4293_4297delGAGAA*
c.4482delG*
c.4379_4380insA
c.4691_4692insTTTC*
c.5142_5144delACG*
c.5337delG*
c.5985_5987delGAG*
c.6652_6653delAT*
1
2 3
4
5 6
7 8 9 10 11 12 13
14
15 17 19 21
23 24 25
26
DNA 5'
3'
A large deletion
from IVS13-1341 to c.3291
Splice site
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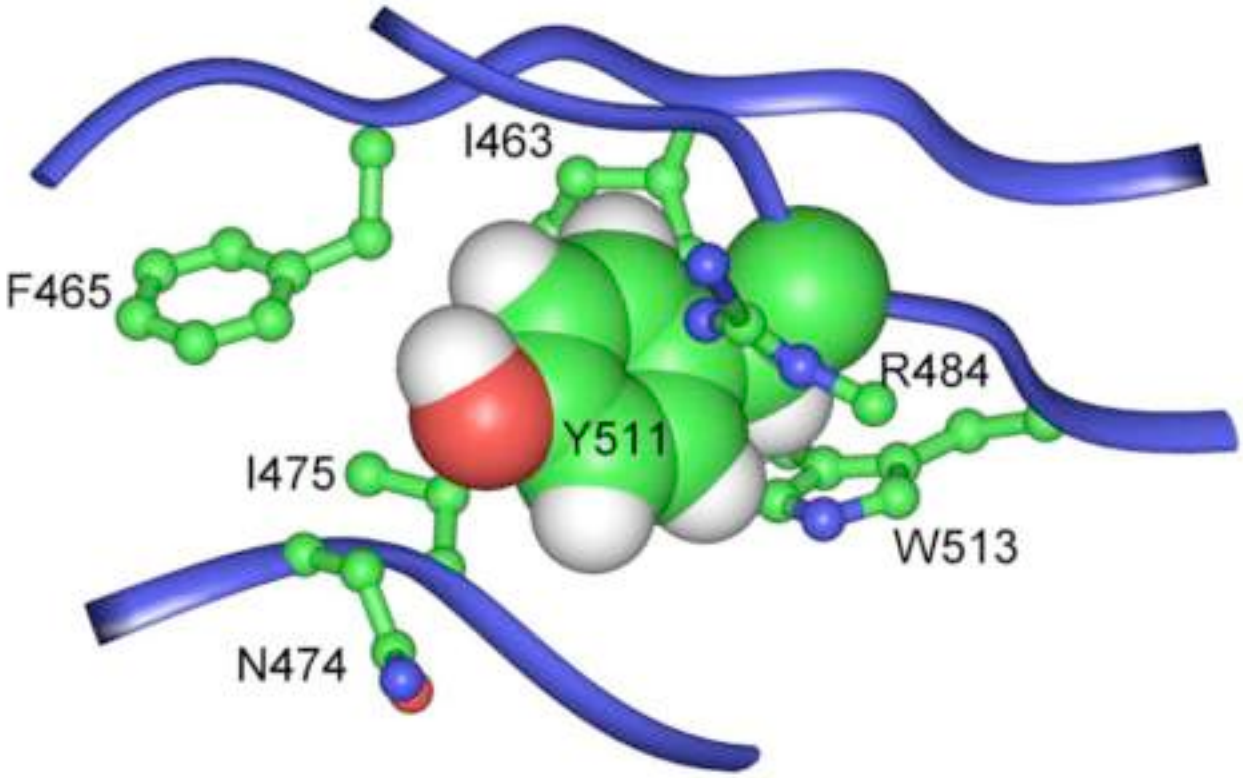
IVS6+1G>A*
IVS11+1G>A*
IVS24-1G>A*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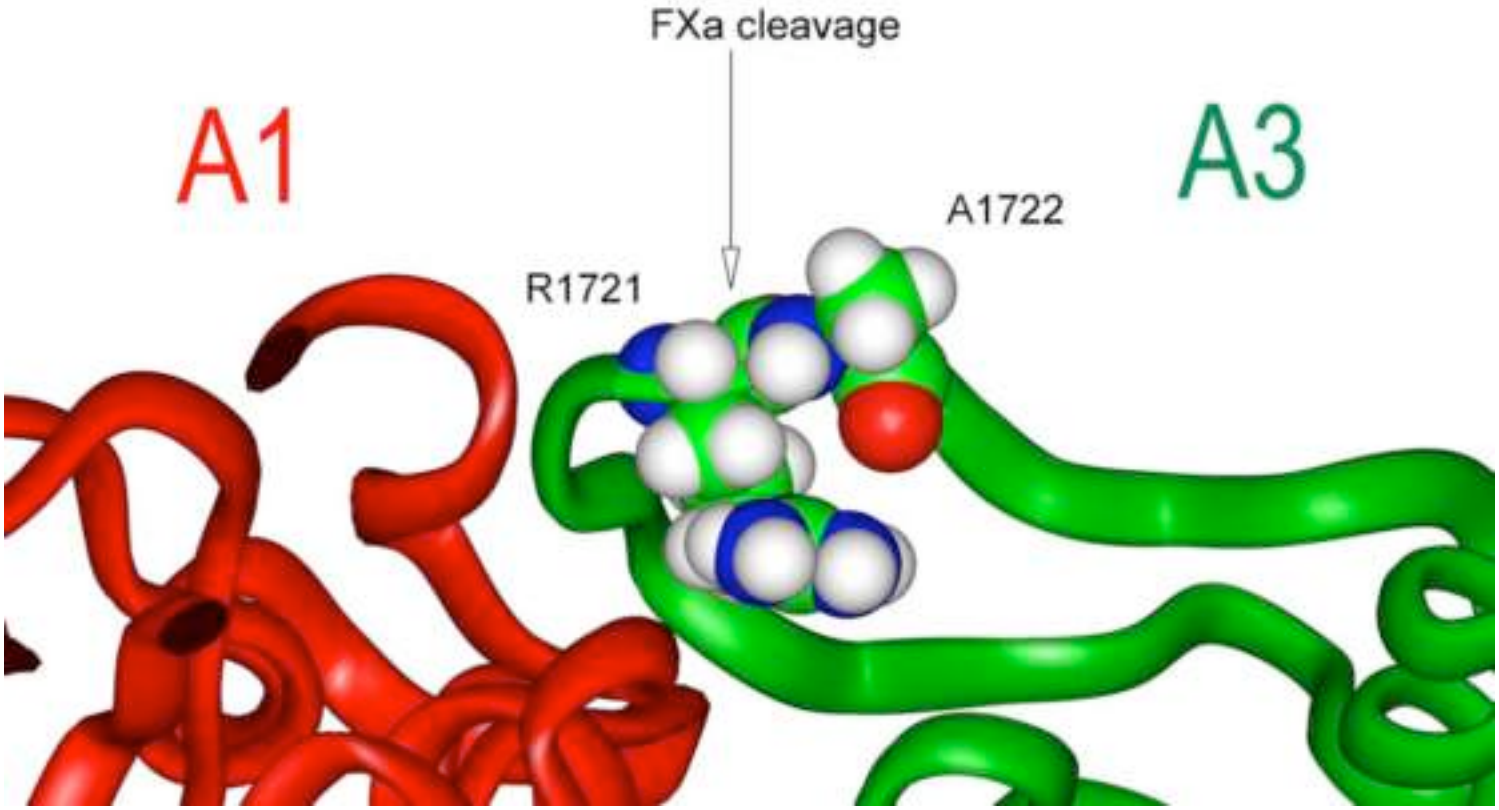
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A3
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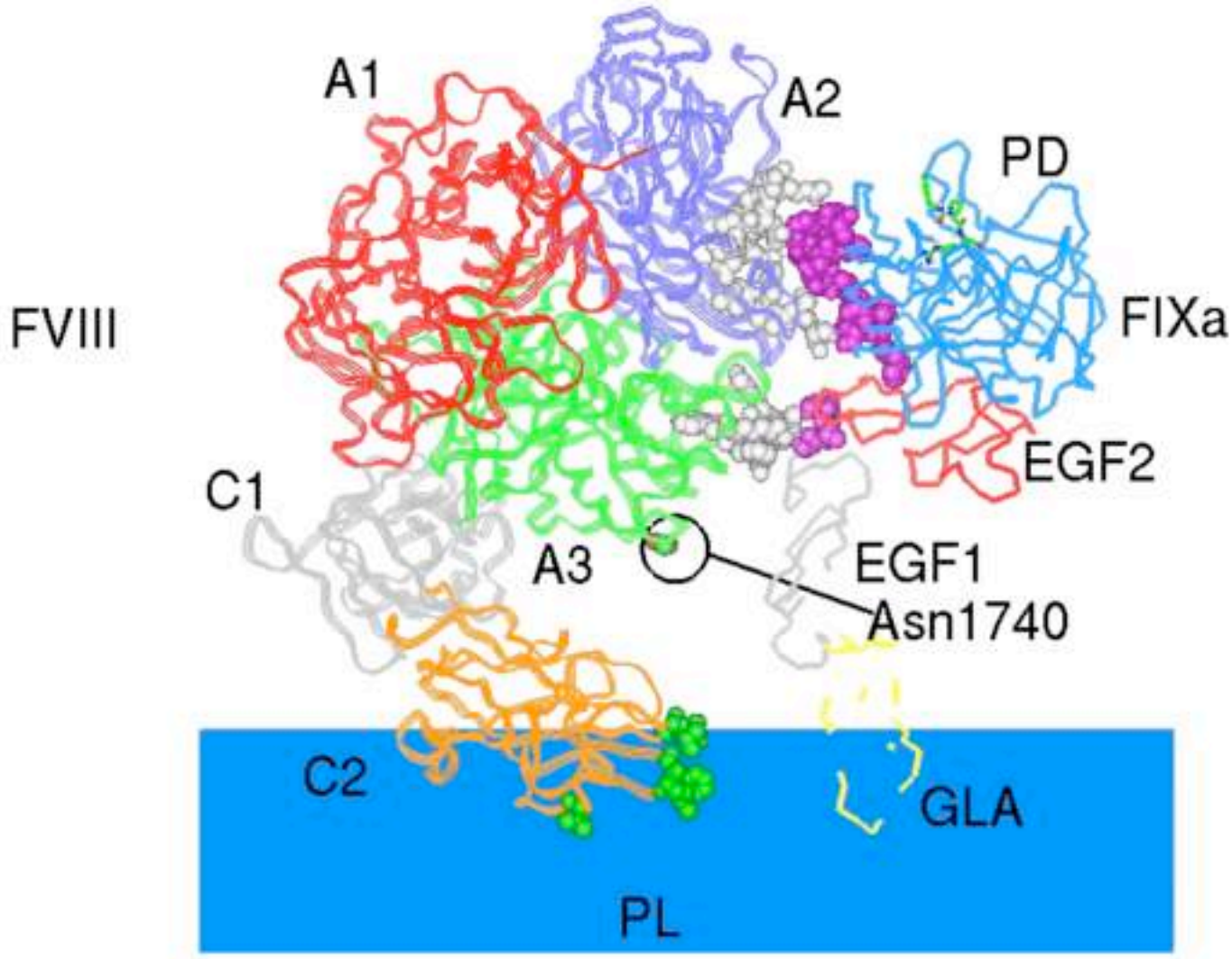
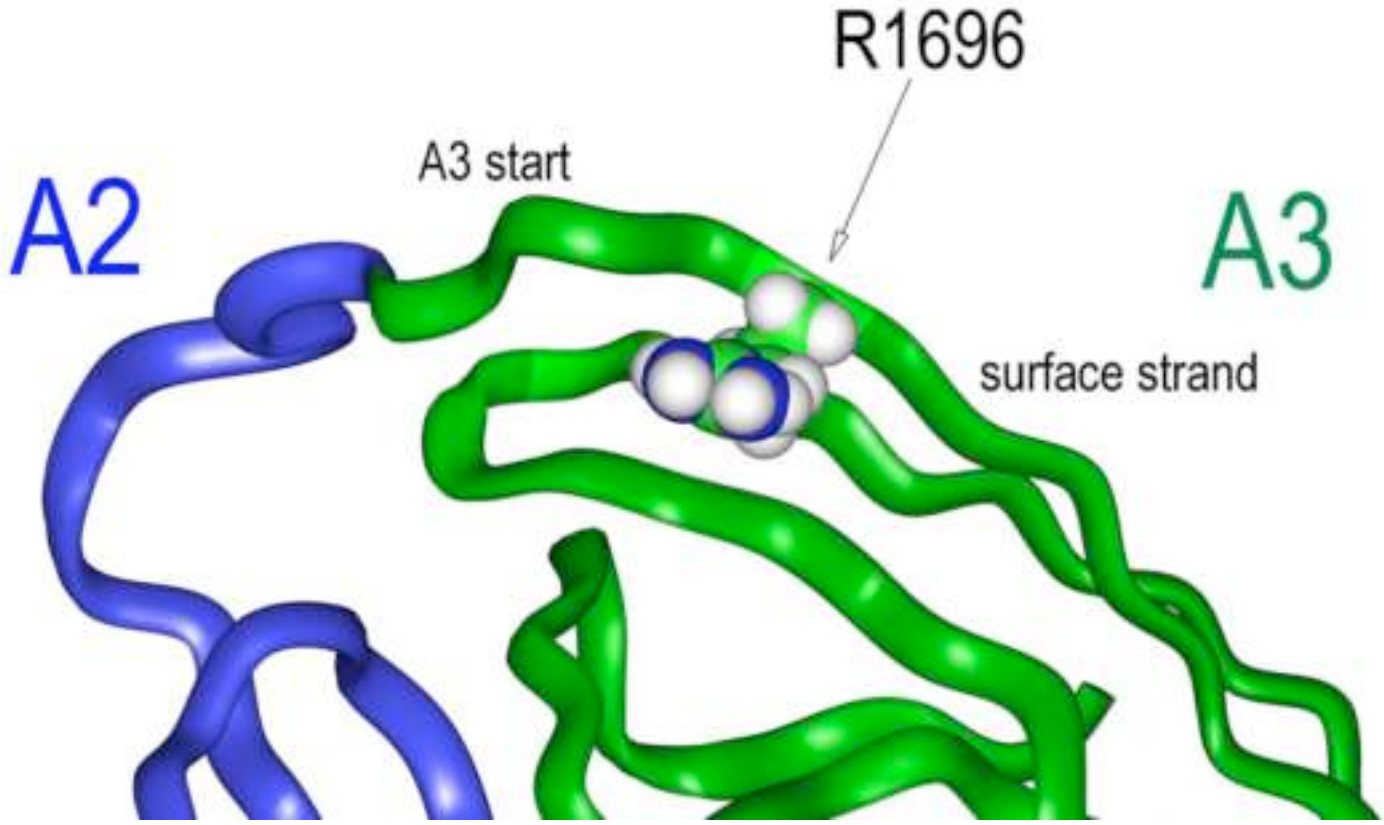
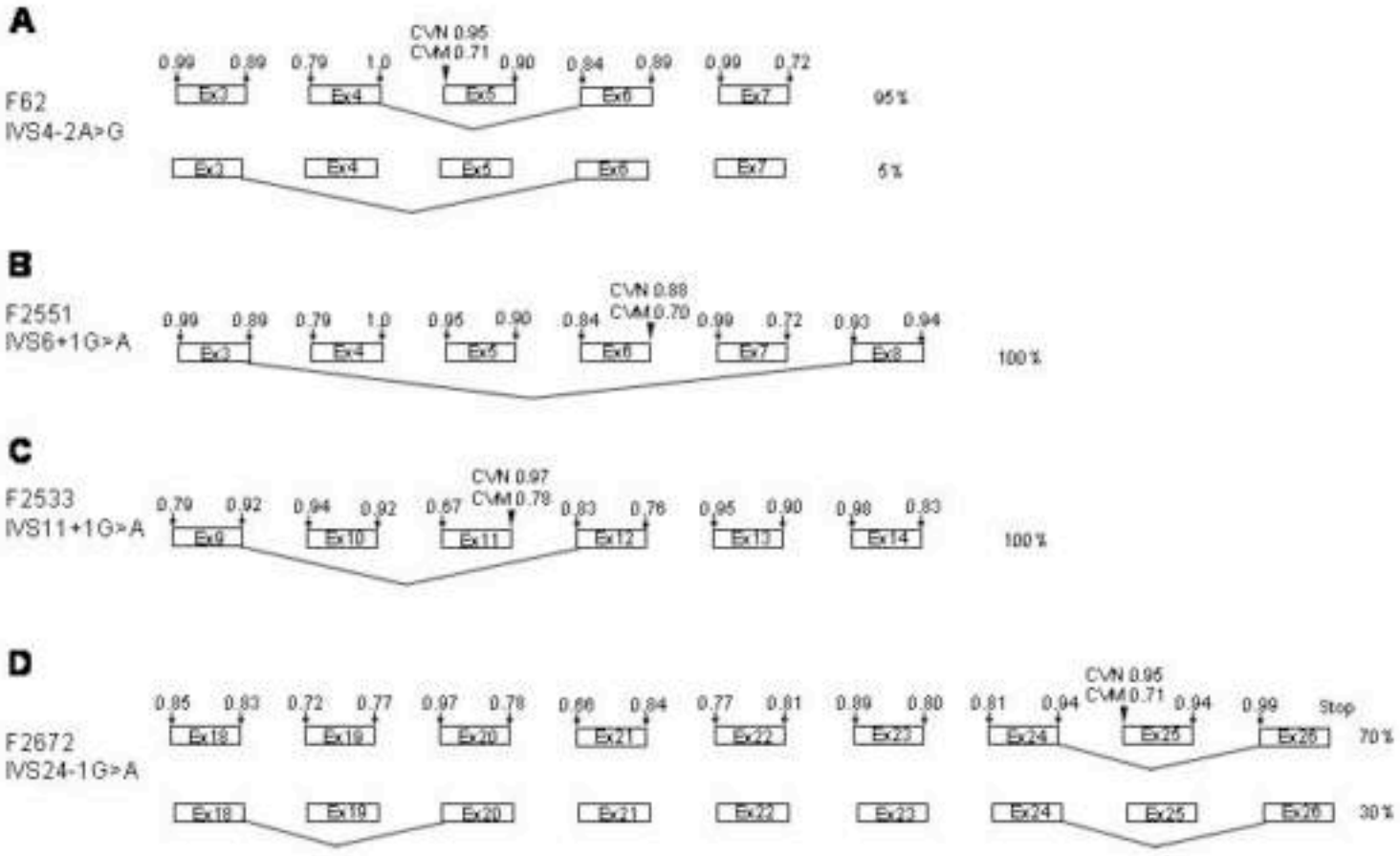
R1721


A1
A2
PD
FVIII
FIXa
EGF2
C1
A3
EGF1
Asn1740
C2
GLA
PL


R1696
A3 start
A2
A3
surface strand


A
F62
IVS4-2A>G
CVN 0.95
CVM 0.71
0.99 0.89 0.79 1.0 0.90 0.84 0.89 0.99 0.72
Ex3 Ex4 Ex5 Ex6 Ex7
95%
Ex3 Ex4 Ex5 Ex6 Ex7
5%
B
F2551
IVS6+1G>A
CVN 0.88
CVM 0.70
0.99 0.89 0.79 1.0 0.95 0.90 0.84 0.99 0.72 0.93 0.94
Ex3 Ex4 Ex5 Ex6 Ex7 Ex8
100%
C
F2533
IVS11+1G>A
CVN 0.97
CVM 0.78
0.79 0.92 0.94 0.92 0.67 0.83 0.76 0.95 0.90 0.98 0.83
Ex9 Ex10 Ex11 Ex12 Ex13 Ex14
100%
D
F2672
IVS24-1G>A
CVN 0.95
CVM 0.71
0.85 0.83 0.72 0.77 0.97 0.78 0.66 0.84 0.77 0.81 0.89 0.80 0.81 0.94 0.94 0.99 Stop
Ex18 Ex19 Ex20 Ex21 Ex22 Ex23 Ex24 Ex25 Ex26
70%
Ex18 Ex19 Ex20 Ex21 Ex22 Ex23 Ex24 Ex25 Ex26
30%