

Clustered *F8* missense mutations cause hemophilia A by combined alteration of splicing and protein biosynthesis and activity

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SUPPLEMENTARY METHODS

Sequence of oligonucleotides used

	Name	sequence 5' -> 3'
Mutagenesis of FVIII(co) cDNA	rFVIII-2013R dir	CTGCCCAGCAAGGCCCGCATCTGGCGGGTG
	rFVIII-2013R rev	CCACCCGCCAGATGCGGGCCTTGCTGGGG
	rFVIII-2016W dir	AAGGCCGGCATCTGGTGGGTGGAGTGTCTA
	rFVIII-2016W rev	TCAGACACTCCACCCACCAGATGCCGGCCT
	rFVIII-2018G dir	GCATCTGGCGGGTGGGGTGTCTGATCGGCA
	rFVIII-2018G rev	TCGCCGATCAGACACCCACCCGCCAGATC
	rFVIII-2038S dir	TTTCTGGTGTACAGCAGCAAGTGCCAGACC
	rFVIII2038S rev	GGGGTCTGGCACTTGCTGCTGTACACCAGA
Creation of coFVIIIΔ19 cDNA	rFVIII delta 19 dir	TGTACCCCGAGTGCCAGACCCCACTGGGCG
	rFVIII delta19 rev	GGTCTGGCACTCGGGGTACAGGTTGTACAG
	rFVIII 10515 dir	CCATCGAACCCCGGAGCTTC
	rFVIII 12278 rev	GACCTGAGGCCGCCAGGC
RT-PCR of transcripts from ectopic F8 mRNA	FVIII ex16 dir	GCCTGGGCTTATTTCTCTGATG
	FVIII ex22 rev1	CCTCGATAAGTCTGCCACTTCT
	FVIII ex17 dir	CCTGCTCATGGGAGACAAGT
	FVIII ex22 rev2	AACTGAGAGATGTAGAGGCT
PCR of the F8 minigene region	F8 int18 Ndel dir	tacatatggagtgccacatctggccaatg
	F8 int19 Ndel rev	tacatatgtctaagttatctggaact
Mutagenesis of the F8 minigene	6037g>a dir	TTACCATCCAAAGCTAGAAATTTGGCGGGTGG
	6037g>a rev	CCACCCGCCAAATTCTAGCTTTGGATGGTAA
	6045g>c dir	CAAAGCTGGAATTTGCCGGGTGGAATGCCT
	6045g>c rev	AAGGCATTCCACCCGGCAAATTCCAGCTTTG
	6046c>t dir	CAAAGCTGGAATTTGGtGGGTGGAATGCCTT
	6046c>t rev	ATAAGGCATTCCACCCaCCAAATTCCAGCTG
	6047g>c dir	AAGCTGGAATTTGGCCGGTGGAAATGCCTTAT
	6047g>c rev	ATAAGGCATTCCACCCGGCCAAATTCCAGCTT

	6049g>a dir	GCTGGAATTTGGCGGATGGAATGCCTTATTG
	6049g>a rev	CAATAAGGCATTCCATCCGCCAAATTCCAGC
	6053a>g dir	GAATTTGGCGGGTGGGATGCCTTATTGGCA
	6053a>g rev	TCGCCAATAAGGCATCCCACCCGCCAAATTC
	6104t>c dir	GCACACTTTTTCTGGCGTACAGCAATAgta
	6104t>c rev	tcacTATTGCTGTACGCCAGAAAAAGTGTGC
	6113a>g dir	TTCTGGTGTACAGCAGTAgtgagtagcaatg
	6113a>g rev	cattgctactcacTACTGCTGTACACCAGAA
	5ss HC F8ex19 dir	TCTGGTGTACAGCACAGgtaagtagcaatgtggca
	5ss HC F8ex19 rev	tgcccacattgctacttacCTGTGCTGTACACCAGA
Creation of the pU1 F8ex19	U1 F8ex19 HC dir	GATCTCATAACTCACTATGCAGGGGAGATAT
	U1 F8ex19 HC rev	GATCATGGTATCTCCCCTGCATAGTGAGTTA
Creation of the pU7F8ex19	U7 F8ex19 dir	ACAGAGGCCTTTCCGCAGCATTCCACCCACCAACAGC AATTTTTGGAG
	SP6 rev	ATTTAGGTGACACTATAGAA
RT-PCR of transcripts from minigenes	alpha 2,3	CAACTTCAAGCTCCTAAGCCACTG
	Bra2	TGATTTAACCAACTTCTGGTGACCGGATCCTA

Exonic and intronic nucleotides are reported in upper and lower cases, respectively. The *NdeI* restriction sites are underlined.