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LETTER TO THE EDITOR

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Three novel F8 mutations in sporadic haemophilia A cases

Rashid Hussain^{1*}, Noman Bin Abid², Sajjad Hussain³, Zeeshan Shaukat³, Mudassir Altaf³, Sara Altaf³ and Gulzar Niazi³

Hemophilia A (HA) is an X-linked hereditary disorder characterized by bleeding of variable severity through mild, moderate to severe owing to large range of mutations in the Factor VIII (F8) gene (Bowen 2002). All kind of F8 mutations, except repeats, have been reported for HA, in total up to 2370 (Human Genome Mutation Database 2005). A preliminary study was conducted in our lab for identification of mutations in F8 gene in Pakistani HA patients. Correlation of F8 mutations with clinical manifestation of HA patients was the main objective of the study. Blood samples were collected from 62 HA patients from all over the Pakistan and clinical history of all HA patients was recorded (only patients frequently visiting medical centers for the replacement of Factor VIII were selected for the study). Genomic DNA was extracted from whole blood by standard organic procedure. Specific primers (Figure 1) were designed using "Primer3" (http://biotools.umass med.edu/bioapps/primer3_www.cgi) to amplify the coding region of F8 gene; amplified products were sequenced by ABI 310 and ABI 3100 sequencer (Applied Biosystems, Carlsbad, CA, USA). The sequencing results were visualized using "Chromas 2.33" software (Applied Biosystems) and mutations were detected using "BLAST" software available on the NCBI website (http: balst.ncbi.nlm.nih.gov/Blast.cgi). Three novel mutations (1 deletion; 2 point mutations) were detected in four sporadic HA patients, all from different ethnic backgrounds (Table 1). The deletion of T in exon 7 within the A1 domain represents a frame-shift change disrupting the protein structure and function, which result in severe manifestation of the disease. A missense point mutation in the A3 domain occurs in codon 1907 at nucleotide number 5720, replacing Serine with Isoleucine, and

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confers a moderate type of severity. It should be noted that Serine is a polar and acidic amino acid while Isoleucine is a nonpolar and basic amino acid. A nonsense point-mutation was found in two unrelated patients in the C3 domain (exon 26) and was correlated with moderate clinical findings. Beside these mutations, 27 common SNPs were also detected in *F8* gene for the studied patients (Table 2). The allelic data and accession numbers of these SNPs were collected from Ensembl Genome Browser (Ensembl 2000). The results of the study will form the basis not only for an enlarged study but also for diagnosis and genetic counseling of classical hemophilia in Pakistan.

Competing interests

The authors declare that they have no competing interests.

Author's contributions

RH managed the project and wrote the paper. NBA, SH, ZS, MA, SA performed experiments. GN designed the project. All authors read and approved the final manuscript.

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Primer	Exon	Primer	Size	Product	Annealing	F8E14.1F	14	GGGAGAGAACCTCTAACAGAA	21	396bp	55.18°C	F8E20R		ATCTGAGATTCTCCACCAGA	20		54.6°
ID				size	Temp.	F8E14.1R		GCATCAACAAATCACTAGAGG	21		54.97°C	1					
F8E1F	1.5.5	CTGCTTCCCACTGATAAAAA	20	1000 C 2000 C 2000	55.55°C	F8E14.2F	14	CCACAATTCCAGAAAATGAC	20	463bp	55.47 °C	F8E21F		CTAGGACTAACCCAGCTGAA	20		54.7°C
F8E1R	1	AGCATCACAACCATCCTAAC	20	457bp	55.06 °C	F8E14.2R		TAATGAACTGGCATACTTGG	20	No. Walter	53.79°C	-	21			210bp	
187 - M			治,就*。	1	6 185 Mg	F8E14.3F	14	TAAATGAGAAACTGGGGACA	20	476bp	55.65°C	F8E21R	1	GAGCTTGCAAGAGGAATAAG	20		54.5°
F8E2F	2	AAGTGTCCACCAAAATGAAC	20	258bp	54.94 °C	F8E14.3R		GCCATCAATGTGAGTCTTTC	20		55.62°C	1 7 10 10 10	Copies				C. Seller
F8E2R		GCACIFITTAACTGCAACCT	20		54.80 °C	F8E14.4F	14	ATGGACCTGCTTTGTTGAC	19	487bp	56.01 °C	F8E22F	22	TTCAGGAGGTAGCACATACA	20	255bp	54.2°
F8E3F	3	TGGAATAACAGGTTTTCTGG	20	250bp	55.23 °C	F8E14.4R		TGACCTTCCACAGATTTTTC	20	-	55.17°C	F8E22R	-	AGTATTCAGGCATTCCCTTT	20	55.4°	
F8E3R	2.202	GCACACACATCTCACTGTTC	20		54.86°C	F8E14.5F		GGATACAAAGGACTCATGGA	20	487bp	54.95°C	VAND? CLAR	0.800				
F8E4F	4	TGTTTCTTTGAGTGTACAGTGG	22	373bp	55.49°C	F8E14.5R 14	TTTGAGAAATGAGCTGTGTG	20		54.89°C	F8E23F	23	GCACAAAGCAAATTAGAAGG	20	20 277bp	55.3°	
F8E4R		TETTTCAGGTGAAGGAACAC	20	9750p	55.24 °C	F8E14.6F	15	GGCATATGCTCCAGTACTTC	20	529bp 54	54.90°C	F8E23R		GTTGAGGGAAGAAGGATATG	20	C. CRANKS	53.8°
F8E5F	5	TCCTCCTAGTGACAATTTCC	20	188bp	54.23 °C	F8E14.6R	14	TGCTGGAAGATGAGAAGAGT	20	02900	55.05 °C	F8E24F	1	CONTOTOTTOTOTTOTO	20		55.1°
F8E5R		GCAGAGGATTTCTTTCAGG	19		54.97 °C	F8E14.7F	-	GAGTCATAGCATCCCTCAAG	20		54.85°C	102241	24	GCATGTCCTTGTGATAACCT	20	277bp	55.1
F8E6F	6	TCATICATGAGACACATGCT	20	2211	54.9°C	F8E14.7R	- 14	CTGTTTGCTTCATTCCACTT	20	492bp	55.45°C	F8E24R		ACCTCAGAAGAAACAGTCAAG	21	53.7°	
F8E6R		ACAGAACTCTGGTGCTGAAT	20	231bp	54.8 °C	F8E14.8F	1.2.00	AGAAGGACCTATTCCCTACG	20	and the	55.02°C	F8E25F	3.3	TTCTGGGAGTAAATGGTGAC	20		55.0°
F8E7F	7	TCCATTCTGTCCTAGCAAGT	20	400bp	54.9°C	F8E14.8R	14.	TGACITCTATTICGGGCTTA	20	369bp	55.23°C		25	THACTORING ACAROLICA	20	295bp	33.0
F8E7R	2 34	CCTTCAGCAACACACTATATTC	22	3. 3.	54.2 °C	F8E14.9F	F	батассатттсоссстбаа	20	-	54.97 °C	F8E25R	13	TTAAGCICTAGGAGAGGTGGT	21		55.0°
F8E8F		GCCTAATATAGCAAGACACTCTG	23	. 358bp	55.2 °C	F8E14.9R	- 14		20	445bp	54.77 °C	F8E26.1F		AGAAGTGAGAAAAGCGTCTG	20	20	54.89
F8E8R	8	TTTTGAGTATGGGGAAGAGA	20		54.8 °C	F8E15F	- 100	TGGTGTCATCATCTGGTAAA	10.400.00	55.3°C	F8E26.1R	26		-	475bp		
F8E9F		ATTITTCTTCCCAACCTCTC	20		54.9°C	Str. Spectrum	15	GAGGATGTGAGGCATTTCTA	20	300bp	55.5°C			GGAAGGAAGGAGTAATCTGG	20	55.38	
1.95.91	9		12.24	302bp		F8E15R	GTGGGAATACATTATAGTCAGC	22		53.1°C	F8E26.2F	253	ATCATCAGTCCTGCATTTCT	20	1.190	54.66	
F8E9R	14. 19	GACAAGGCTGAATTATGAGG	20	200	54.9°C	F8E16F		GGGATGTAAACCCTAAGGAC	20		55.1 °C		26			480bp	
F8E10F		GGCCACTTTTATTTATCTGG	20		54.1 °C	F8E16R	16	AGCTTCTTATTGCACGTAGG	389bp		54.9°C	F8E26.2R		GTGCCCCTCATAATGACTAA	20	0	55.17
F8E10R	10	CTGGAGAAAGGACCAACATA	20	284bp	55.2 °C	F8E17F	-		10		55.2°C	F8E26.3F		ACAATCTGCAAAATGGAGAG	20		55.37
F8E11F		CAGATTTGTAGAACCCTTGC	20		55.0°C	110	17	TGAGAAATCCACTCTGGTTC	20	371bp		F8E26.3R	26			491bp	
	11		122	361bp		F8E17R		CCTGGATCAAGTCTCATTTG	20	00-5	55.6°C	FoE20.5K		GGGAGAGAGAGTAAACTGAGTGC	21		55.70
F8E11R		AAGGGGACATACACTGAGAA	20		S TREE Res 1	F8E18F	18	ATATCTGTGGGAGTGGAATC	20	389bp	53.81 °C	F8E26.4F	26	GATGACATTAGGCTTCTAAAGG	22	1071-2	54.95
F8E12F	12	GACTGCTAGCTCCTACCTGA	20	262bp 56.0 °C	54.8 °C	F8E18R		TCTGCTTTGATCACTGATTG	20		54.79°C	F8E26.4R		TEACCATOTOCTOTTTTTCA	20	49/00	54.85
8E12R	12	TCITTATTCACCACCCACTG	20		56.0 °C	F8E19F		ACCAATGTATCTCATGCTCA	20	1.15	539°C	A Carl	5	TTAGGATCTCCTGTTTTCCA	20	1.332	54.85
F8E13F		TCTCTTCCTGGGAATAAGAT	20	2021	53.0°C	F8E19R	19 F8E19R	AGGCTGAGTAGGTAGGGAAC	20	226bp	55.1°C	F8E26.5F	26	GGCTGGAGACAAGGATAAGT	20	600bp	55.90
8E13R	13	13 ATACGAATGGCTAGTGAAGC 20		393bp	54.7°C	F8E20F	20	GCTGAATTTTGTGCACTTCT	20	199bp	55.6°C	F8E26.5R		CAGTGCCCCTATTTGTTTTA	20	1	55.43
<u>61 - 13</u>	R. Bar		같은 명	66.12	e 1967. S		l	er i stationer i stati			55.0°C					1	

Table 1 Novel mutations in F8 gene

Age/Sex	Severity	Exon	Nucleotide change	Amino acid change	Codon/Codon no.	Nucleotide genome ref./cDNA ref.	Affected Domain
4 yr /male	Severe	7	Deletion of T	Frame-shift	CTC \rightarrow C-C/ 318	159197688/953	A1
35 yr / male	Moderate	17	$G \mathop{\rightarrow} T$	$Ser \to lle$	AGC \rightarrow ATC/ 1907	154132724/5720	A3
15 & 19 yr /male	Moderate	26	$C \mathop{\rightarrow} A$	$Tyr \rightarrow Termination$	TAC \rightarrow TAA/ 2324	154065994/6972	C2

yr (years).

Table 2 Common SNPs in F8 gene (exonic region)

Sr. #	Patient	Exon	SNP ambiguity	SNP	Codon	Codon#	Comments	Accession number
1	All 62 Samples	2	W: A/T	A/A	<u>GA</u> T	75	European = T/T	rs1800288
2	All 62 Samples	7	K: G/T	G/G	TG <u>G</u>	274	European = C/C; Spanish Caucasians = C(0.995)/A(0.005); African American, Chinese, Southeast Asia, Mexican Indian = C/A	rs34371500
3	All 62 Samples	8	R: G/A	G/G	C <u>G</u> C	391	Ancestral: G	rs137852364
4	All 62 Samples	8	Y: T/C	T/T	TCA	392	European = C/C	rs28933669
5	All 62 Samples	8	Y: C/T	C/C	TCA	392	?	rs28933668
6	All 62 Samples	8	K: T/G	T/T	ATT	405	European = A/A	rs28933670
7	All 62 Samples	8	R: A/G	A/A	GAG	409	?	rs28933671
8	All 62 Samples	9	K: G/T	T/T	TTG	431	Ancestral: G	rs28933672
9	All 62 Samples	9	R: A/G	A/A	AAA	444	Ancestral: G	rs28937272
10	All 62 Samples	9	W: T/A	T/T	TAC	450	Ancestral: A	rs111033616
11	All 62 Samples	10	R: G/A	G/G	CGT	503	Ancestral: A	rs35383156

12	All 62 Samples	12	Y: T/C	T/T	CTT	622	Ancestral: T	rs1800290
13	All 62 samples	15	R: G/A	G/G	CAG	1764	Ancestral: A	rs5986891
14	All 62 samples	16	R: G/A	G/G	ATG	1842	European = G/G	rs28943674
15	All 62 samples	16	Y: C/T	C/C	CCC	1844	European = C/C	rs28933675
16	All 62 samples	16	M: A/C	A/A	ACT	1845	?	rs28933676
17	All 62 samples	16	Y: C/T	C/C	GCC	1853	European = C/C	rs28933677
18	All 62 samples	17	D: G/A/T	G/G	GAT	1865	Not Available	Cl076951
19	All 62 samples	17	R: A/G	A/A	C <u>A</u> C	1867	Ancestral: G	rs28933679
20	All 62 samples	17	S: C/G	C/C	C <u>C</u> C	1873	European = G/G	rs28933680
21	All 62 samples	17	R: G/A	G/G	GAG	1904	European = C/C	rs28933681
22	All 62 samples	17	S: G/C	G/G	T <u>G</u> C	1922	European = G/G	rs4384155
23	All 62 samples	17	S: C/G	C/C	TGC	1922	European = C/C	rs4520342
24	All 62 samples	18	R: A/G	A/A	AAT	1940	?	CM083806
25	All 62 samples	18	D: G/A/T	G/G	CGA	1960	?	rs28937294
26	All 62 samples	18	R: G/A	G/G	GGC	1967	?	rs111033615
27	All 62 samples	24	Y: C/T	C/C	TAC	2214	Ancestral: C	rs1800296

Table 2 Common SNPs in F8 gene (exonic region) (Continued)