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LETTER TO THE EDITOR Open Access

Three novel *F8* mutations in sporadic haemophilia A cases

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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.umassmed.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 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 ID	Exon	Primer	Size	Product size	Annealing Temp.
F8E1F	1	CTGCTTCCCACTGATAAAAA	20	457bp	55.55 °C
F8E1R		AGCATCACAACCATCCTAAC	20		55.06 °C
F8E2F	2	AAGTGTCCACCAAAATGAAC	20	258bp	54.94 °C
F8E2R		GCACTTTTAACTGCAACCT	20		54.80 °C
F8E3F	3	TGGAATAACAGGTTTTCTGG	20	250bp	55.23 °C
F8E3R		GCACACACATCTCACTGTTC	20		54.86°C
F8E4F	4	TGTTTCTTTGAGTGTACAGTGG	22	373bp	55.49°C
F8E4R		TCTTTCAGGTGAAGGAACAC	20		55.24°C
F8E5F	5	TCCTCCTAGTGACAATTTCC	20	188bp	54.23 °C
F8E5R		GCAGAGGATTTCTTTCAGG	19		54.97°C
F8E6F	6	TCATTCATGAGACACATGCT	20	231bp	54.9 °C
F8E6R		ACAGAACTCTGGTGCTGAAT	20		54.8 °C
F8E7F	7	TCCATTCTGTCCTAGCAAGT	20	400bp	54.9 °C
F8E7R		CCTTCAGCAACACACTATATTC	22		54.2 °C
F8E8F	8	GCCTAATATAGCAAGACACTCTG	23	358bp	55.2 °C
F8E8R		TTTTGAGTATGGGGAAGAGA	20		54.8 °C
F8E9F	9	ATTTTTCTTCCCAACCTCTC	20	302bp	54.9 °C
F8E9R		GACAAGGCTGAATTATGAGG	20		54.9 °C
F8E10F	10	GGCCACTTTTATTTATCTGG	20	284bp	54.1 °C
F8E10R		CTGGAGAAAGGACCAACATA	20		55.2 °C
F8E11F	11	CAGATTTGTAGAACCCTTGC	20	361bp	55.0 °C
F8E11R		AAGGGGACATACACTGAGAA	20		54.6 °C
F8E12F	12	GACTGCTAGCTCCTACCTGA	20	262bp	54.8 °C
F8E12R		TCTTTATTCACCACCCACTG	20		56.0 °C
F8E13F	13	TCTCTTCCTGGGAATAAGAT	20	393bp	53.0 °C
F8E13R		ATACGAATGGCTAGTGAAGC	20		54.7 °C
F8E14.1F	14	GGGAGAGAACCTCTAACAGAA	21	396bp	55.18 °C
F8E14.1R		GCATCAACAAATCACTAGAGG	21		54.97 °C
F8E14.2F	14	CCACAATTCCAGAAAATGAC	20	463bp	55.47 °C
F8E14.2R		TAATGAACTGGCATACTTGG	20		53.79 °C
F8E14.3F	14	TAAATGAGAAACTGGGGACA	20	476bp	55.65 °C
F8E14.3R		GCCATCAATGTGAGTCTTTC	20		55.62 °C
F8E14.4F	14	ATGGACCTGCTTTGTTGAC	19	487bp	56.01 °C
F8E14.4R		TGACCTTCCACAGATTTTTC	20		55.17 °C
F8E14.5F	14	GGATACAAAGGACTCATGGA	20	487bp	54.95 °C
F8E14.5R		TTTGAGAAATGAGCTGTGTG	20		54.89 °C
F8E14.6F	14	GGCATATGCTCCAGTACTTC	20	529bp	54.90 °C
F8E14.6R		TGCTGGAAGATGAGAAGAGT	20		55.05 °C
F8E14.7F	14	GAGTCATAGCATCCCTCAAG	20	492bp	54.85 °C
F8E14.7R		CTGTTTGCTTCATTCCACTT	20		55.45 °C
F8E14.8F	14	AGAAGGACCTATTCCCTACG	20	369bp	55.02 °C
F8E14.8R		TGACTTCTATTTCGGGCTTA	20		55.23 °C
F8E14.9F	14	GATACCATTTTGTCCCTGAA	20	445bp	54.97 °C
F8E14.9R		TGGTGTCATCATCTGGTAAA	20		54.75 °C
F8E15F	15	GAGGATGTGAGGCATTTCTA	20	300bp	55.3 °C
F8E15R		GTGGGAATACATTATAGTCAGC	22		53.1 °C
F8E16F	16	GGGATGTAAACCCTAAGGAC	20	389bp	55.1 °C
F8E16R		AGCTTCTTATTGCACGTAGG	20		54.9 °C
F8E17F	17	TGAGAAATCCACTCTGGTTC	20	371bp	55.2 °C
F8E17R		CCTGGATCAAGTCTCATTTG	20		55.6 °C
F8E18F	18	ATATCTGTGGGAGTGGAATC	20	389bp	53.81 °C
F8E18R		TCTGCTTTGATCACTGATTG	20		54.79 °C
F8E19F	19	ACCAATGTATCTCATGCTCA	20	226bp	53..9°C
F8E19R		AGGCTGAGTAGGTAGGGAAC	20		55.1 °C
F8E20F	20	GCTGAATTTTGTGCACTTCT	20	199bp	55.6 °C
F8E20R		ATCTGAGATTCTCCACCAGA	20		54.6 °C
F8E21F	21	CTAGGACTAACCCAGCTGAA	20	210bp	54.7 °C
F8E21R		GAGCTTGCAAGAGGAATAAG	20		54.5 °C
F8E22F	22	TTCAGGAGGTAGCACATACA	20	255bp	54.2 °C
F8E22R		AGTATTCAGGCATTCCCTTT	20		55.4 °C
F8E23F	23	GCACAAAGCAAATTAGAAGG	20	277bp	55.3 °C
F8E23R		GTTGAGGGAAGAAGGATATG	20		53.8 °C
F8E24F	24	GCATGTCCTTGTGATAACCT	20	277bp	55.1 °C
F8E24R		ACCTCAGAAGAAACAGTCAAG	21		53.7 °C
F8E25F	25	TTCTGGGAGTAAATGGTGAC	20	295bp	55.0 °C
F8E25R		TTAAGCTCTAGGAGAGGTGGT	21		55.0 °C
F8E26.1F	26	AGAAGTGAGAAAAGCGTCTG	20	475bp	54.89 °C
F8E26.1R		GGAAGGAAGGAGTAATCTGG	20		55.38 °C
F8E26.2F	26	ATCATCAGTCCTGCATTTCT	20	480bp	54.66 °C
F8E26.2R		GTGCCCCTCATAATGACTAA	20		55.17 °C
F8E26.3F	26	ACAATCTGCAAAATGGAGAG	20	491bp	55.37 °C
F8E26.3R		GGGAGAGAGTAAACTGAGTGC	21		55.70 °C
F8E26.4F	26	GATGACATTAGGCTTCTAAAGG	22	497bp	54.95 °C
F8E26.4R		TTAGGATCTCCTGTTTTCCA	20		54.85 °C
F8E26.5F	26	GGCTGGAGACAAGGATAAGT	20	600bp	55.90 °C
F8E26.5R		CAGTGCCCCTATTTGTTTTA	20		55.43 °C

Figure 1 Primers used in the study.

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 → C-C/ 318	159197688/953	A1
35 yr / male	Moderate	17	G → T	Ser → Ile	AGC → ATC/ 1907	154132724/5720	A3
15 & 19 yr /male	Moderate	26	C → A	Tyr → Termination	TAC → 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	GAT	75	European = T/T	rs1800288
2	All 62 Samples	7	K: G/T	G/G	TGG	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	CGC	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

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

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	CI076951
19	All 62 samples	17	R: A/G	A/A	CAC	1867	Ancestral: **G**	rs28933679
20	All 62 samples	17	S: C/G	C/C	CCC	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	TGC	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