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ORIGINAL PAPER - SUPPLEMENTARY MATERIAL

CFTR Exon 10 deleterious mutations in patients with congenital bilateral absence of vas deferens in a cohort of Pakistani patients

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Table S1.
Sequences of primers used for the PCR amplification, amplicon size along with its position on human genome.

Sr. #	Primer	Sequence	Amplified genomic region	Product size
01	EX10-CFTR-FWD EX10-CFTR-REV	5'-AGTGTAATGGATCATGGGC-3' 5'-CTTCCAGCACTACAAACTAG-3'	chr7:117548511 - 117548903	393bp
02	PR-CFTR-FWD PR- CFTR-REV	5'-TGGACCTAAAGAGAGGCC-3' 5'-ACCTCTGCATGGTCTCTC-3'	chr7:117479636 - 117480104	469bp

Table S2.
Identified mutations in CFTR-Exon10.

Sr.#	Patient's ID	Mutation position on genome	Nucleotide change	Amino acid change	Mutation type
01	HWP3	Chr7: 117,548,798	T-C	V456A	Missense
02	HWP4	Chr7: 117,548,798	T-C	V456A	Missense
03	HWP6	Chr7: 117,548,822	A-G	K464E	Missense
04	HWP12	Chr7: 117,548,798	T-C	V456A	Missense
05	HWP13	Chr7: 117,548,742	T-G	G437G	Silent
06	HWP14	Chr7: 117,548,798	T-C	V456A &	Missense
		Chr7: 117,548,724	T-C	S431S	Silent

Figure S1.
Agarose gel electrophoresis of DNA products: (a) Extracted genomic DNA from selected patients resolved using 1% agarose. (b) Validation of 469bp PCR products amplified from promoter region of CFTR (PR-CFTR). (c) Validation of 393bp PCR products amplified from Exon 10 of CFTR (EX10-CFTR).

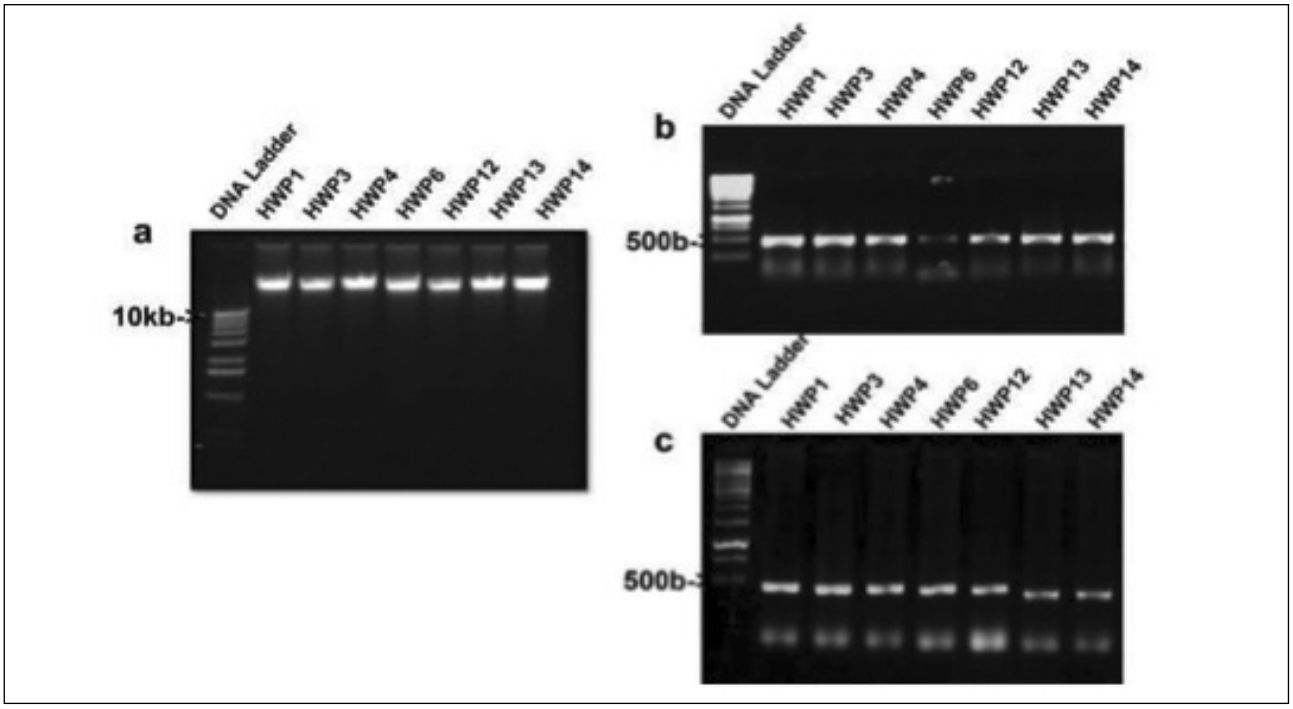


Figure S2.
Sequence alignment using BioEdit of all the processed samples. Red arrows indicate position of V456A mutation.

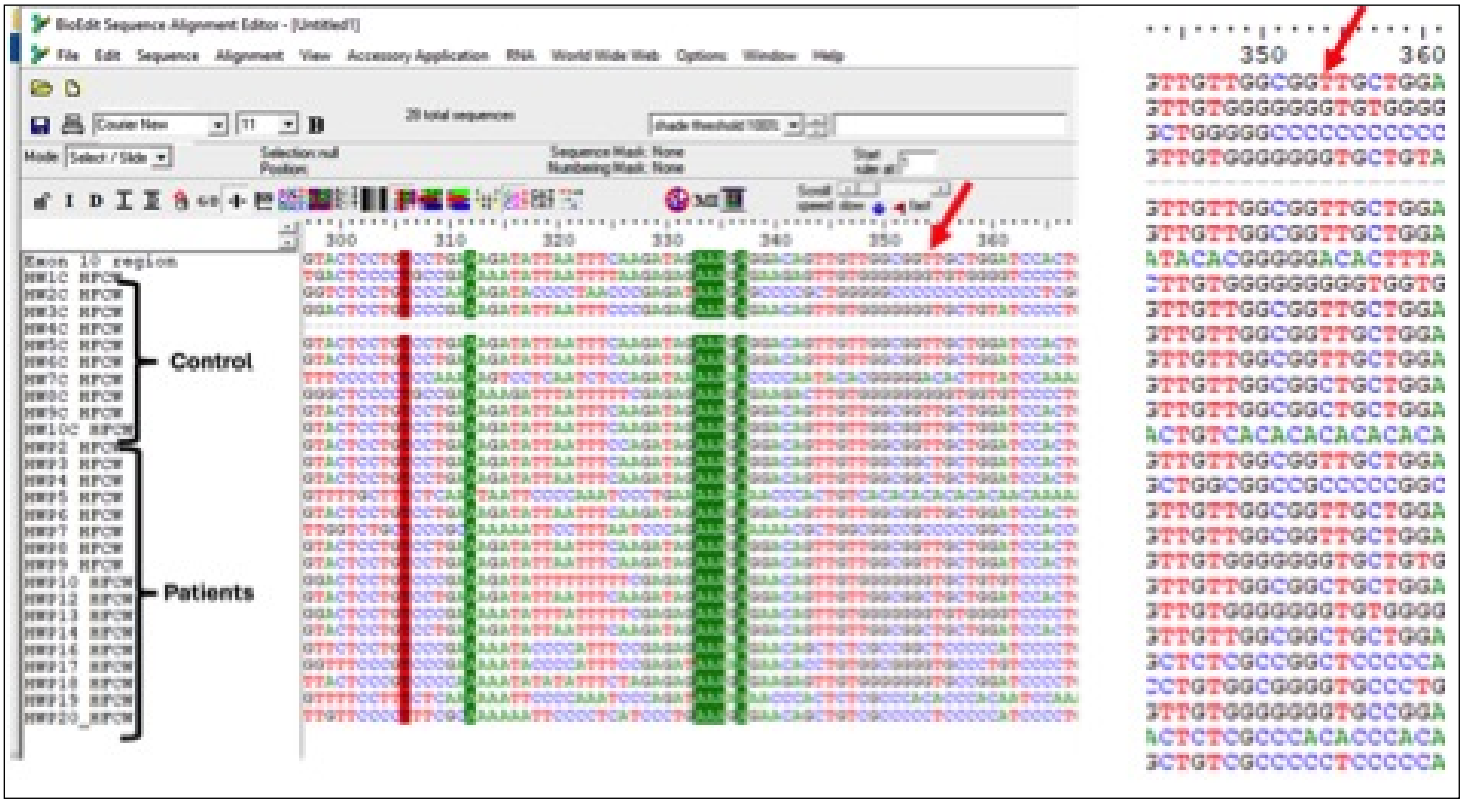


Figure S3.
Sequence alignment using BioEdit of all the selected samples. Red arrows indicate position of V456A mutation.

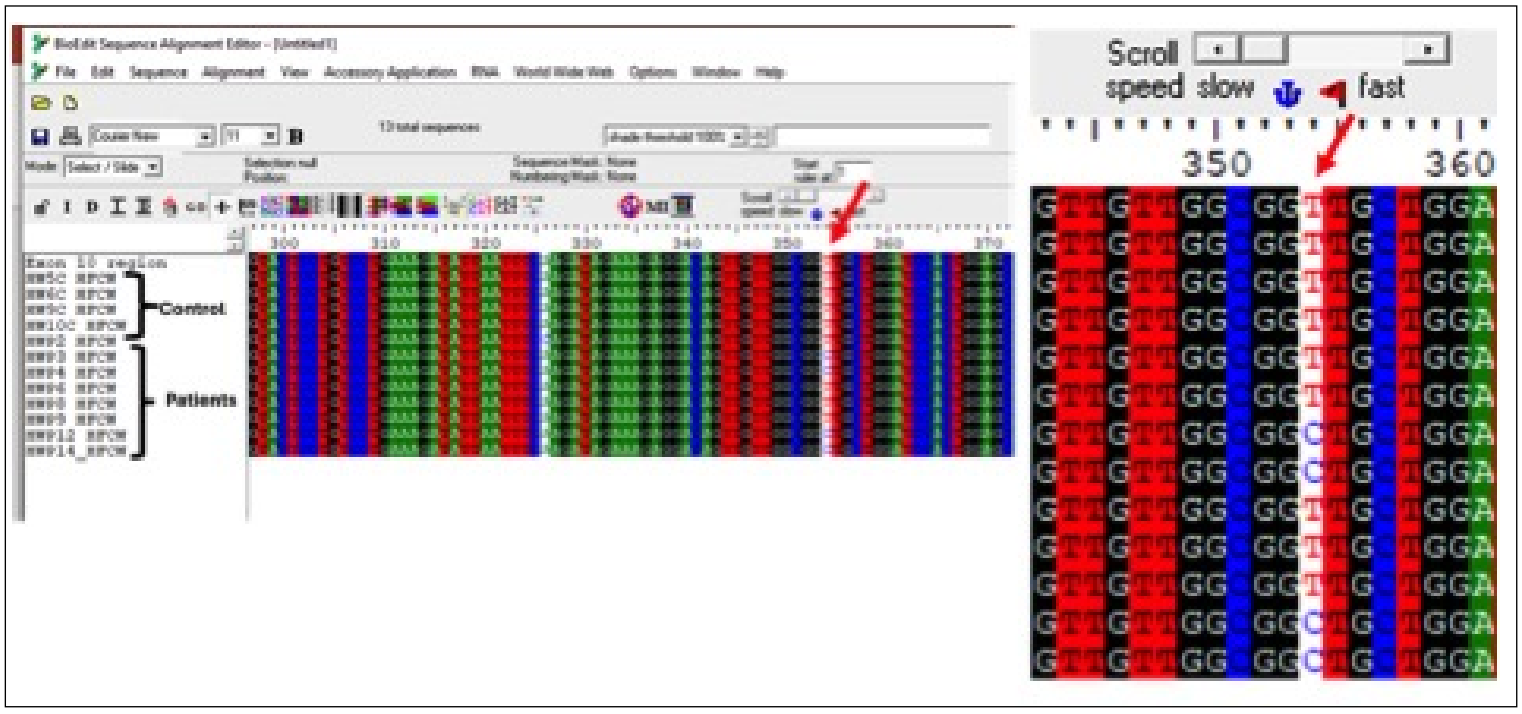


Figure S4.
Structure of the CFTR protein (PDB ID-6O1V) is displayed. a) The orthoscopic view of CFTR protein. b) The functional site in Exon 10 is shown in cyan c) The selected region of Exon 10 and identified mutations (V456A, K464E, and G437G) are represented as sticks. d) Mutation V456A e) Mutation K464E f) Mutation G437G. Each mutation is labeled with the corresponding amino acid change and highlights the spatial arrangement of critical regions in the protein using PyMol software.

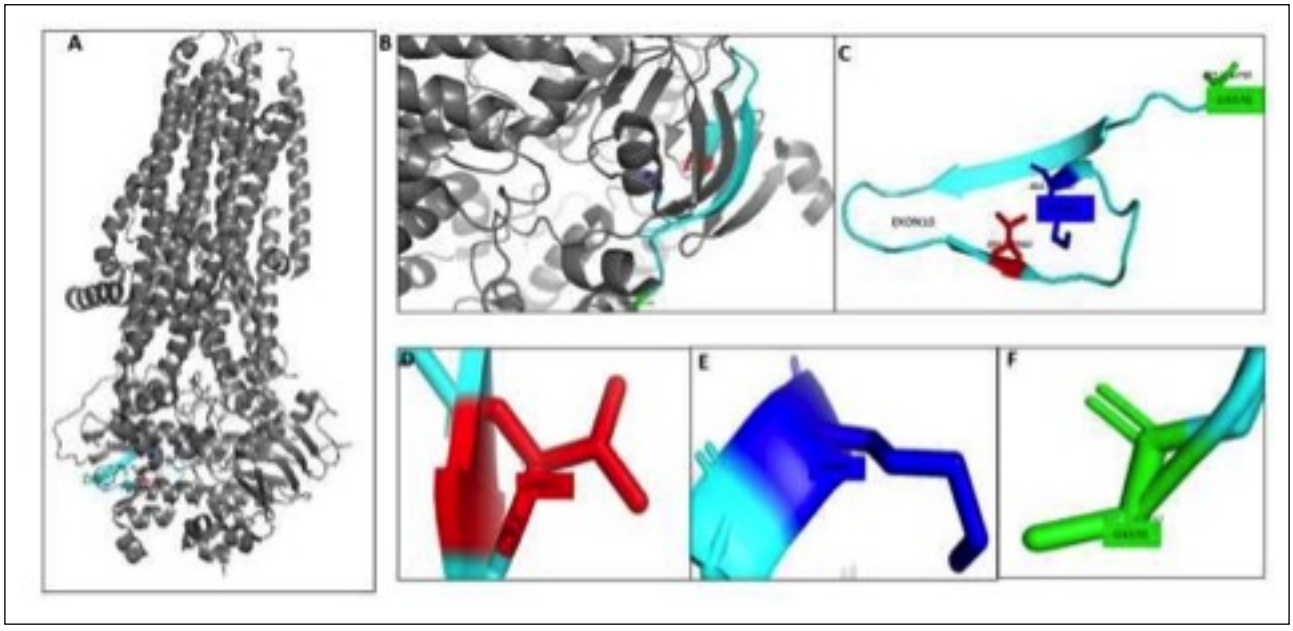
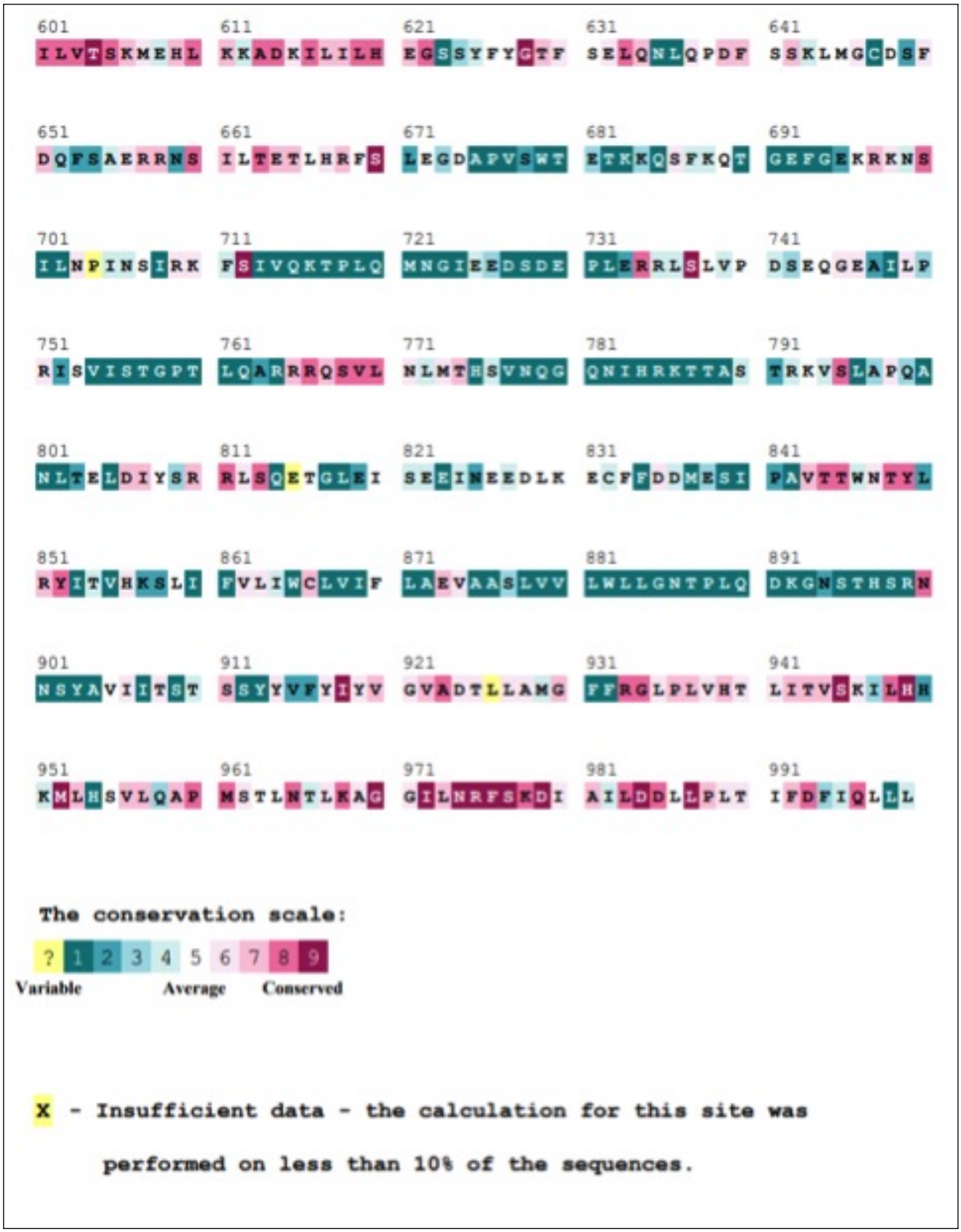


Figure S5.
ConSurf analysis.


601 611 621 631 641
ILVTSKMEHL KKADKILILH EGSSYFYGTF SELQNLQPDF SSKLMGCDSF
651 661 671 681 691
DQFSAERRNS ILTETLHRFS LEGDAPVSWT ETKKQSFKQT GEFGEKRKNS
701 711 721 731 741
ILNPINSIRK FSIVQKTPLQ MNGIEEDSDE PLERRLSLVP DSEQGEAILP
751 761 771 781 791
RISVISTGPT LQARRRQSVL NLMTHSVNQG QNIHRKTTAS TRKVSLAPQA
801 811 821 831 841
NLTELDIYSR RLSQETGLEI SEEINEEDLK ECFFDDMESI PAVTTWNTYL
851 861 871 881 891
RYITVHKSLI FVLIWCLVIF LAEVAASLVV LWLLGNTPLQ DKGNSTHSRN
901 911 921 931 941
NSYAVIITST SSYYVFYIYV GVADTLLAMG FFRGLPLVHT LITVSKILHH
951 961 971 981 991
KMLHSVLQAP MSTLNTLKAG GILNRFSKDI AILDDLLPLT IFDFIQLLL
The conservation scale:
? 1 2 3 4 5 6 7 8 9
Variable
Average
Conserved
X - Insufficient data - the calculation for this site was performed on less than 10% of the sequences.