Supplementary Material

Material and Methods

Ethical compliance

The study was approved by an ethics committee. All subjects provided written informed consent for the study, which was conducted in accordance with the Declaration of Helsinki.

Next generation sequencing

Genomic DNA samples of the patients were extracted from peripheral blood using the QIAamp DNA Blood Mini Kit (Qiagen; Hilden, Germany).

The mutational screening was carried out in patient and her parents using both a Whole Exome Sequencing (WES) and a targeted resequencing (TR) for the only *CFTR* gene.

For WES, genomic DNA was enriched for library preparation according to the Twist Human Core Exome+RefSeq Panel for Enrichment (Twist). Sequencing was performed on NextSeq 500 (Illumina Inc., San Diego, CA) and reads were aligned to the human reference genome GRCh37/hg19. Variant calling was performed using GATK v4.1.2 HaplotypeCaller (EnGenome, Pavia, Italy) while the eVAI software (EnGenome, Pavia, Italy) - based on the American College of Medical Genetics and Genomics (ACMG) guidelines - was used for the annotation and prioritization of the variants

The TR approach was conducted with the Ion PGM™ platform (IPGM; Thermo Fisher Scientific; Waltham, MA), as previously indicated (1). All variants identified were confirmed in the proband and in her parents by Sanger sequencing, as previously described (2).

Bioinformatic analyses

The missense variant was searched in the following annotation databases: Single Nucleotide Polymorphism Database (dbSNP; http://www.hgmd.cf.ac.uk/ac/index.php), Genome Aggregation Database (gnomAD; https://gnomad.broadinstitute.org), Human Gene Mutation Database (HGMD; http://www.hgmd.cf.ac.uk/ac/index.php; “DM”), and CFTR2 (https://cftr2.org/; “CF-causing”). The effect of the amino acid change was evaluated by means of several pathogenicity prediction programs, including Combined Annotation Dependent Depletion (CADD score=29.1; https://cadd.gs.washington.edu/), Mutation Taster (“Disease causing”, score=56;

http://www.mutationtaster.org/), Mutation Assessor ("Medium", FI score=2.755; http://mutationassessor.org/r3/), Polyphen-2 ("Probably damaging", score=1.000; http://genetics.bwh.harvard.edu/pph2/) and Sorting Intolerant From Tolerant (SIFT, "Not tolerated"; https://sift.bii.a-star.edu.sg/).

CNV detection

A statistical analysis consisting of two normalization steps was performed to detect copy number variations (CNV) (3). An intra-sample normalization was determined by dividing the total number of reads of each amplicon by the total reads average obtained from the same library. An inter-sample normalization was then calculated by dividing the intra-sample normalization of each amplicon by the average of all the intra-sample normalization of this amplicon from 10 control samples. Inter-sample normalization ratios between 0.7 and 1.3 were regarded as a wild type condition with two copies of single amplicons.

Long range PCR as well GAP PCR were performed in order to characterize the deletion identified in patient.

Point mutations and deletion characterization

The CFTR region affected by the mutations was amplified by a standard PCR from patient's genomic DNA using two specific primers (Table S1). Then the PCR product was cloned into a pJET1.2 cloning vector (Thermo scientific) and used to transform OneShot^{TM} Top10 competent cells (Thermo Fisher Scientific; Waltham, MA). The colonies were screened by Sanger sequencing.

Long PCR was performed using the GoTaq^{TM} Long PCR Master Mix (Promega; Madison, WI) according to manifacturer's instruction (Table S1).

PCR products were bidirectionally sequenced using an ABI 3100 automated sequencer (Applied Biosystem, Forster City, CA). Nucleotide A of the ATG translation initiation start site of the *CFTR* cDNA in GenBank sequence NM_000492.3 is indicated as nucleotide +1.

SNP array analysis

SNP array was performed using the Human OmniExpressExome-8 Bead Chip (Illumina Inc., San Diego, CA) containing 960,919 loci. Data analysis was performed using Illumina's Genome Studio software v2.0.1. The copy number variants, mapped to the human reference genome hg19, were

annotated with UCSC RefGene. Allele detection, genotype calling, B allele frequencies (BAFs) and log R ratios were obtained using the Genome Studio software.

References

1. Faleschini M, Melazzini F, Marconi C, Giangregorio T, Pippucci T, Cigalini E, et al. ACTN1 mutations lead to a benign form of platelet macrocytosis not always associated with thrombocytopenia. Br J Haematol. 2018;183(2):276-88.
2. Bottega R, Pecci A, De Candia E, Pujol-Moix N, Heller PG, Noris P, et al. Correlation between platelet phenotype and NBEAL2 genotype in patients with congenital thrombocytopenia and α-granule deficiency. Haematologica. 2013;98(6):868-74.
3. Nicchia E, Greco C, De Rocco D, Pecile V, D'Eustacchio A, Cappelli E, et al. Identification of point mutations and large intragenic deletions in Fanconi anemia using next-generation sequencing technology. Mol Genet Genomic Med. 2015;3(6):500-12.

Table S1

	Primer name	Sequence (5'>3')	Lenght (bp)	Temperature (T°)
Cloning Primers	CFTR F	CCTGAGCGTGATTTGATAATG	302	60
	CFTR R	TTGGGTAGTGTGAAGGGTTC		
Sequencing Primers	11F	CCTGAGCGTGATTTGATAATG	295	62
	11R	TTGGGTAGTGTGAAGGGTTC		
Long PCR	i19F_long	AAGTTCCCATCTCTGGTAGC	6896 (wt); 561 (del)	60
	i21R_long	GCCTTCCAAGATTCATCAAG		
GAP PCR	i19_gap	ATTTCACAGGCAGGAGTCCA	101 (wt)	60
	20R_gap	CAGAGTTTCAAAGTAAGGCTGCC		
	i19_gap	ATTTCACAGGCAGGAGTCCA	175 (del)	60
	i21R_gap	GGGAGTAGTGTCATGGAAGCA		