Supplementary Information

SUPPLEMENTARY METHODS

Blood sample

Peripheral venous blood (5 ml) was collected into EDTA tubes for the following experiments. Written informed consent was obtained from cases in our study. The study was approved by the institutional ethics committee.

Whole-genome sequencing

Genomic DNA was isolated from peripheral blood samples of the subjects using a whole blood DNA purification kit (QIAGEN). Whole-genome sequencing (WGS) was performed on the two brothers. First, 1.0 μg genomic DNA was sheared into an average size of 350 bp fragments by Covaris S220 sonicator. Second, ends of the gDNA fragments were repaired; 3' ends were adenylated. Both ends of the gDNA fragments were ligated at the 3' ends with paired-end adaptors (Illumina) with a single 'T' base overhang and purified using AMPure SPRI beads from Agencourt. Then, the size distribution and concentration of the libraries were determined using Agilent 2100 Bioanalyzer and qualified by real-time PCR (2 nM), respectively. At last, DNA libraries were sequenced on Illumina HiSeq X according to the manufacturer's instructions for paired-end 150 bp reads.

Reads were mapped to the reference genome (UCSC hg19) by the Burrows-Wheeler Aligner (BWA) software to get the original mapping result in BAM format, and duplicates were marked and removed using Picard (http://broadinstitute. github.io/picard/index.html). ANNOVAR was performed for functional annotation through a variety of databases, such as dbSNP, 1000 Genome, ExAC, and HGMD. After filtering, the retained nonsynonymous SNVs were submitted to PolyPhen-2, SIFT, MutationTaster, and CADD for functional prediction.

Whole DNA methylome analysis

Illumina Infinium HD Methylation 850 K arrays were used to perform on blood cells of the two brothers following the Illumina Infinium HD Methylation protocol. Infinium Human Methylation 850 BeadChip includes 851,764 cytosine positions of the human genome covering >14,000 genes. The beadchip was scanned by an Illumina iScan. Raw data were further analyzed using Illumina BeadStudio software and the Methylation Module add-in (Illumina, San Diego, CA, USA). The relative methylation level of each CG site was evaluated as the ratio of normalized methylated signal intensity to the sum of methylated and unmethylated signal intensities using GenomeStudio software.

RNA sequencing

A total amount of 3 μg RNA per sample was used as input material for the RNA sample preparations. Sequencing libraries were generated using NEBNext® UltraTM RNA Library Prep Kit for Illumina® (NEB, USA) following the manufacturer's recommendations and index codes were added to attribute sequences to each sample.

The clustering of the index-coded samples was performed on a cBot Cluster Generation System using TruSeq PE Cluster Kit v3 - cBot - HS (Illumina) according to the manufacturer's instructions. After cluster generation, the library preparations were sequenced on an Illumina Hiseq platform and 125 bp/150 bp paired-end reads were generated. Index of the reference genome was built using STAR and paired-end clean reads were aligned to the reference genome using STAR (v2.5.1b). HTSeq v0.6.0 was used to count the reads numbers mapped to each gene. FPKM of each gene was calculated based on the length of the gene and reads count mapped to this gene. Differential expression analysis of two conditions/groups (two biological replicates per condition) was performed using the DESeq 2 R package (1.10.1). Gene Ontology (GO) and KEGG enrichment analysis of differentially expressed genes was implemented by the clusterProfiler R package. GATK2 (v3.2.1) software was used to perform SNP calling and SnpEff software was used to annotation for the Variable site.

rMATS (3.2.1) software was used to analysis the ASevent. SOAPfuse (1.27) software was used for fusion analysis.

qPCR

Total RNA was extracted using TRIzol regent (Invitrogen, Carlsbad, CA, 15596-026) and converted to cDNA (RevertAid First-Strand cDNA Synthesis Kit, ThermoFisher, Waltham, MA, K1622). qPCR was performed using SYBR Premix Ex Taq II (TaKaRa Bio, Dalian, China, RR820A), and reactions were run using a Bio-Rad iCycler RT-PCR Detection System. The primers that were used for qPCR are listed as follow:

SCNN1B	5'GTTCCTGCTCACCCTGCTCT 3'	5' GGGTTCCGTTCATCAATAAGG 3'
CA12	5'TAAAGGAACAGCCTTCCAGC 3'	5' TGCTTGTTGGCAGACAGATT 3'
GAPDH	5' AAGGTCGGAGTCAACGGATTTG 3'	5' CCTGGAAGATGGTGATGGGATT 3'

Western blot

The proteins of the blood samples were separated on 10% SDS–polyacrylamide gels and transferred onto a polyvinylidene difluoride (PVDF) membrane (Millipore, Temecula, CA, IPVH00010) and detected with specific appropriate primary antibodies and horseradish peroxidase-conjugated secondary antibodies. The antibodies used in the study were CFTR (Millipore, 2796322), CA12 (Santa Cruz Biotechnology, sc-374313), SCNN1B (Santa Cruz Biotechnology, sc-25354), and GAPDH (Abcam, Cambridge, MA, ab8245).

Statistical analysis

The statistical analyses were performed using SPSS 17.0 software (IBM Company, Chicago, IL, USA). Student's *t*-test was used to compare the observed indexes between the experimental groups. A $P < 0.05$ was considered significant.