SUPPLEMENTARY MATERIAL

Supplementary Material 1. Methods and steps of our genetic analysis

First, we extracted a genomic DNA sample from the leukocytes in the peripheral blood of our patient by using an Agilent DNA extraction kit (Cat# 200600; Agilent Technologies, Santa Clara, CA, USA). We then used the gene panels to confirm the absence of aberrant short-tandem-repeat expansion disorders including SCA1, SCA2, SCA3 (also called Machado–Joseph disease), SCA6, SCA8, SCA12, SCA17, and dentatorubral–pallidoluysian atrophy. Subsequently, we performed whole exome sequencing with exome capture by using a Human Core Exome Kit (Twist Bioscience, South San Francisco, CA, USA), followed by massive parallel sequencing with a NovaSeq 6000 platform (Illumina, San Diego, CA, USA). We then aligned and mapped the sequencing data to the reference genome by using Burrows-Wheeler Aligner ("BWA-MEM -t 32 -c 100 -M -R"). We also performed Indel realignment and deduplication by using Picard tools (version 1.9; http://broadinstitute.github.io/picard/). Next, we genotyped short insertion/deletion mutations and single nucleotide variants by using the Genome Analysis Toolkit. To catalog and annotate the detected variations, we used HaplotypeCaller, ANNOVAR, and the VarSome database (https://varsome.com/, accessed on February 25, 2022). Subsequently, we filtered variations with a homopolymer length greater than 6 (and synonymous substitutions) or variations that were common (> 0.5%) in dbSNP150 (https://www.ncbi.nlm.nih.gov/snp/), HapMap, the 1000 Genomes Project (https://www.internationalgenome.org/), and the Genome Aggregation Database (https://gnomad.broadinstitute.org/).

Finally, we confirmed the causal variations identified using Sanger sequencing of our patient's pedigree members. The primers of the candidate variations were designed using Primer 3 (https://primer3.ut.ee/). The PCR primer sequences at the genomic DNA level are shown in Supplementary Table 2 in the online-only Data Supplement. Genomic DNA was PCR-amplified using 2X ACE Taq-Plus Master Mix (ACE Biolabs, Taoyuan, Taiwan). The PCR products were sequenced using Applied Biosystems BigDye Terminator sequencing chemistry and then run on an ABI3730xl genetic analyzer according to the manufacturer's instructions (Applied Biosystems, Foster City, CA, USA). Sequence analysis was performed using Lasergene (DNASTAR, Madison, WI, USA).