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SUPPLEMENTARY MATERIAL

1. Whole-Exome Sequencing

Genomic DNA was collected from the peripheral blood of the patient, her parents, and healthy siblings using a QIAsymphony DNA Midi Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. A whole-exome library for each sample was prepared using the Agilent SureSelect v6 Capture Kit (Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer's instructions. The libraries were then sequenced on an Illumina HiSeq2500 platform in 151-bp paired-end mode. After adapter trimming with Cutadapt 1.7.1, the reads were mapped to the hs37d5 reference genome using BWA-MEM (https://broadinstitute.github.io/picard/) and processed with SAMtools 1.3 (http://samtools.sourceforge.net/) and Picard 2.1.1 (http://picard.sourceforge.net/). Variant calling was performed using the Genome Analysis Toolkit (GATK) 3.5 (https://software.broadinstitute.org/gatk/) following the recommended best practices (including base quality score recalibration and local realignment around indels). Grepwalk (https://github.com/glires/grepwalk) was also incorporated into the pipeline for additional analyses. The resulting variant frequencies were compared with those in the integrated Japanese Genome Variation Database (iJGVD; https://ijgvd.megabank.tohoku.ac.jp).

2. Preparation of PBMCs and T cells

Peripheral blood mononuclear cells (PBMCs) were isolated from the whole blood of the patient and healthy controls using Histopaque-1077 (Sigma-Aldrich, St. Louis, MO, USA). T cells of the patient and healthy controls were isolated from PBMCs using CD3 MicroBeads (130-050-101l; Miltenyi Biotec, Bergisch Gladbach, Germany) and a MiniMAC Separator with an MS Column, according to the manufacturer's instructions.

3. Protein Extraction, Antibodies, and WB Analysis

After washing with cold PBS, the PBMCs were incubated with RIPA Lysis and Extraction Buffer (Thermo Fisher Scientific, Waltham, MA, USA) with 1% protease inhibitor cocktail (Merck KGaA, Darmstadt, Germany) for 10 minutes on ice. Thereafter, they were homogenized with a 27-G needle and centrifuged at 15,000 ×*g* for 15 minutes at 4°C; the supernatant was then collected as whole-cell lysate.

Cytoplastic protein from the T cells was extracted using NE-PER Nuclear and Cytoplasmic Extraction Reagents (Thermo Fisher Scientific), according to the manufacturer's instructions.

Western blot (WB) was performed using whole-cell lysates of PBMCs and cytoplasmic extracts of T cells. Primary antibodies were diluted as described below. Following incubation with horseradish peroxidase-conjugated secondary antibodies, the membrane blots were visualized using ECL Select Western Blotting Detection Reagent (RPN2235; Cytiva, Marlborough, MA, USA) and imaged using ImageQuant LAS4000 (GE Healthcare, Chicago, IL, USA). The band intensities of TRAF3 and ACTB were digitized using ImageQuant LAS4000 with the ImageQuant TL Analysis Toolbox (GE Healthcare). The expression of TRAF3 was then normalized to that of ACTB and compared with that of the controls for each analysis. Antibodies against β-actin (M177-3, mouse monoclonal [WB 1:1,000]; MBL, Tokyo, Japan) and TRAF3 (#4729, mouse monoclonal [WB 1:1,000]; Cell Signaling Technology, Danvers, MA, USA) were purchased from MBL and Cell Signaling Technology, respectively. Horse anti-mouse IgG (horseradish peroxidase-linked antibody #7076) and sheep anti-mouse IgG (horseradish peroxidase-linked whole Ab NA931) were used as the secondary antibodies and purchased from Cell Signaling Technology and GE Healthcare, respectively.