

METHODS

Western blotting

AK2 protein expression was detected by using a polyclonal antibody (Proteintech, Chicago, Ill) using extracts from magnetically purified CD3⁺ T cells (STEMCELL Technologies, Vancouver, British Columbia, Canada) and fibroblast lines. Beta actin was detected by using a loading control kit (Cell Signaling Technology, Beverly, Mass).

Assessment of maternal engraftment

DNA extracted from magnetically separated populations was subjected to short tandem repeat analysis as previously described.^{E1} Flow-based analysis was performed by using antibody to HLA-A2 (BB7.2, Biolegend, San Diego, Calif), which distinguished the mother (HLA-A2, -A33) from the patient (HLA-A23, -A33).

Assessment of T-cell receptor diversity

Percentage of expression of TCRV β families in CD3⁺, CD4⁺, and CD8⁺ cells was detected by using specific mAbs for 24 families according to the manufacturer's specifications (Beckman Coulter, Brea, Calif) and

counterstaining with fluorochrome-conjugated antibodies to CD3 (UCHT1), CD4 (OKT4), and CD8a (SK1) (Biolegend, San Diego, Calif).

Disruption of mitochondrial membrane potential in T lymphocytes

CD3⁺ T cells positively selected from Ficoll-purified PBMC using anti-CD3⁺-conjugated magnetic beads (Miltenyi Biotec, Cambridge, Mass) were incubated at 37°C in 5% CO₂ in complete RPMI medium (4% FCS, 100 unit/mL penicillin, 100 ug/mL streptomycin, and 2 mM L-glutamine) with or without 6 uM staurosporin (Sigma-Aldrich, St Louis, Mo) for 3, 6, and 9 hours. MitroPT TMRE dye (ICT945 AbD Serotec) 150 nM was added during the last 20 minutes of culture, and intracellular TMRE staining and CD3 surface expression were detected by using FACS Calibur (BD Biosciences, San Jose, Calif). Data was analyzed by using FlowJo software (Treestar, Ashland, Ore).

REFERENCE

- E1. Pai S-Y, Levy O, Jabara HH, Glickman JN, Stoler-Barak L, Sachs J, et al. Allogeneic transplantation successfully corrects immune defects, but not susceptibility to colitis, in a patient with nuclear factor-kappaB essential modulator deficiency. *J Allergy Clin Immunol* 2008;122:1113-8.e1.



FIG E1. Patient exhibited desquamative erythroderma.

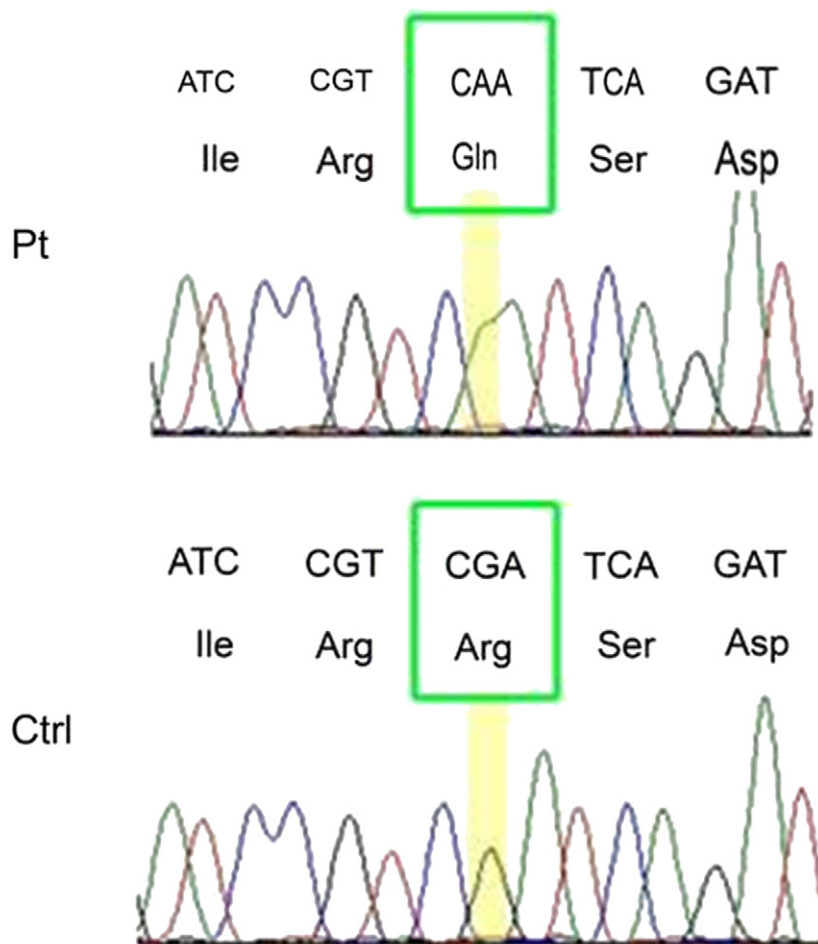


FIG E2. Analysis using genomic DNA from fibroblasts and CD3⁺ lymphocytes demonstrating a missense mutation in AK2 (c.524 G>A, p.R175Q).