

Letter to the Editor

First reported case of Omenn syndrome in a patient with reticular dysgenesis

To the Editor:

Reticular dysgenesis (RD) is a rare form of autosomal-recessive severe combined immunodeficiency characterized by lack of circulating T lymphocytes, severe congenital neutropenia, and sensorineural deafness.^{1,2} The disease is caused by mutations in the gene encoding adenylate kinase 2 (AK2), a mitochondrial protein important for regulating intracellular levels of adenosine diphosphate and maintaining mitochondrial membrane potential. A similar function is also mediated by the cytoplasmic enzyme AK1. While most tissues express both AK1 and AK2 enzymes, neutrophils, T lymphocytes, and cells of the stria vascularis in the inner ear uniquely express AK2, thus explaining the RD phenotype.^{3,4} Omenn syndrome (OS) has been described in association with several genetic defects responsible for severe combined immunodeficiency and results from residual development of oligoclonal T lymphocytes that undergo peripheral expansion and infiltrate various tissues including the skin, gut, liver, and lymphoid organs.⁵ We report the first case of OS in a patient with RD.

A male infant of Kuwaiti origin was born to consanguineous parents who had a previously affected daughter with RD due to a homozygous missense AK2 mutation (c.524 G>A, p.R175Q). The sister's first hematopoietic stem cell transplant from cord blood with reduced intensity conditioning failed, but she later had a successful matched related transplant with conditioning consisting of busulfan and cyclophosphamide.⁶ At birth, the infant was vigorous with an otherwise unremarkable physical examination; however, laboratory studies demonstrated leukopenia, absence of neutrophils, and undetectable IgA and IgM levels (Table I), and a chest radiograph lacked a thymic shadow.

Immunologic evaluation at our institution at 6 weeks of age revealed undetectable T-cell receptor excision circles with detectable RNase P, near-absence of natural killer cells, and B-cell lymphopenia. The T-cell count was slightly reduced, composed entirely of CD45RA-cells. T-lymphocyte proliferation to mitogens was decreased (Table I). Auditory brain stem response-evoked potential testing documented profound hearing loss. A diagnosis of RD was presumed, and mutation analysis by using genomic DNA derived from fibroblasts confirmed homozygosity for the same AK2 mutation as the infant's sister. The patient was started on prophylactic antibiotics and antifungal medications along with intravenous gammaglobulin. At 8 weeks, the infant developed desquamative erythroderma, pachydermia (see Fig E1 in this article's Online Repository at www.jacionline.org), diarrhea, and generalized lymphadenopathy. The CD3⁺ T-cell count increased to 10,032 cells/ μ L (Table I). Treatment with methylprednisolone 1 mg/kg given twice daily and cyclosporine was started; improvement was noted clinically, with laboratory studies documenting decreased circulating T lymphocytes (Table I). The infant underwent a 9/10 HLA-A mismatched unrelated donor bone marrow transplant at 3 months of age, with conditioning consisting of 4 days of busulfan given every 6 hours with area under the curve dosing targeted to 800 to 1200 μ mol*min/L, cyclophosphamide 50 mg/kg daily for 4 days, and equine antithymocyte globulin 30 mg/kg daily for 3 days. Cyclosporine and 1 mg/kg per day of methylprednisolone were continued for graft versus host prophylaxis. There was no evidence for liver involvement secondary to the OS as the pretransplant liver function test results were normal. Vitamin E and ursodiol were administered for veno-occlusive disease prophylaxis. He achieved neutrophil engraftment on day +16, with 100% donor chimerism in the peripheral blood documented on day +21. The child unfortunately developed severe veno-occlusive disease with portal

TABLE I. Hematologic and immunologic characteristics of the patient

	Birth	6 wk	8 wk	10 wk s/p MP, CsA	Normal values
WBC (K cells/ μ L)	0.350	3.36	8.33	1.43	7.20-18.00
ANC (K cells/ μ L)	N/A	0	0.08	0.09	2.57-7.54
ALC (K cells/ μ L)	N/A	3.16	7.96	1.43	3.40-7.60
IgA (mg/dL)	0	<7	-	-	6-50
IgM (mg/dL)	0	<5	-	-	15-70
IgE (IU/mL)	-	1	10	-	0-30
CD3 ⁺ (cell/ μ L) (%)	-	1918 (71)	10032 (92)	788 (86)	2500-5500 (53-84)
CD4 ⁺ (cell/ μ L) (%)	-	1414 (53)	8094 (74)	516 (56)	1600-4400 (35-64)
CD8 ⁺ (cell/ μ L) (%)	-	526 (19)	2069 (19)	293 (32)	560-1700 (12-28)
CD16 ⁺ 56 ⁺ (cell/ μ L) (%)	-	36 (1)	75 (1)	25 (3)	170-1100 (4-18)
CD19 ⁺ (cell/ μ L) (%)	-	703 (26)	734 (7)	93 (10)	300-2000 (6-32)
Naive CD3 ⁺ CD4 ⁺ (%)	-	0	-	-	64-95
Memory CD3 ⁺ CD4 ⁺ (%)	-	100	-	-	2-22
Naive CD3 ⁺ CD8 ⁺ (%)	-	0	-	-	80-99
Memory CD3 ⁺ CD8 ⁺ (%)	-	100	-	-	1-9
PHA (counts/min)	-	35205	-	-	>27846*
TREC (copies/ μ L)	-	0	-	-	<252
RNase P (copies/ μ L)	-	21359	-	-	-

ANC, Absolute neutrophil count; ALC, absolute lymphocyte count; CsA, cyclosporine A; MP, methylprednisolone; s/p, status post; TREC, T-cell receptor excision circle, measured as described from dried blood spot with RNase P control gene determination⁷; WBC, white blood cell.

*5th percentile for normal adult donors in this laboratory. The 50th percentile for normal adult donors in this laboratory is 141,254 counts/min.

Normal values are provided in the rightmost column.^{8,9}

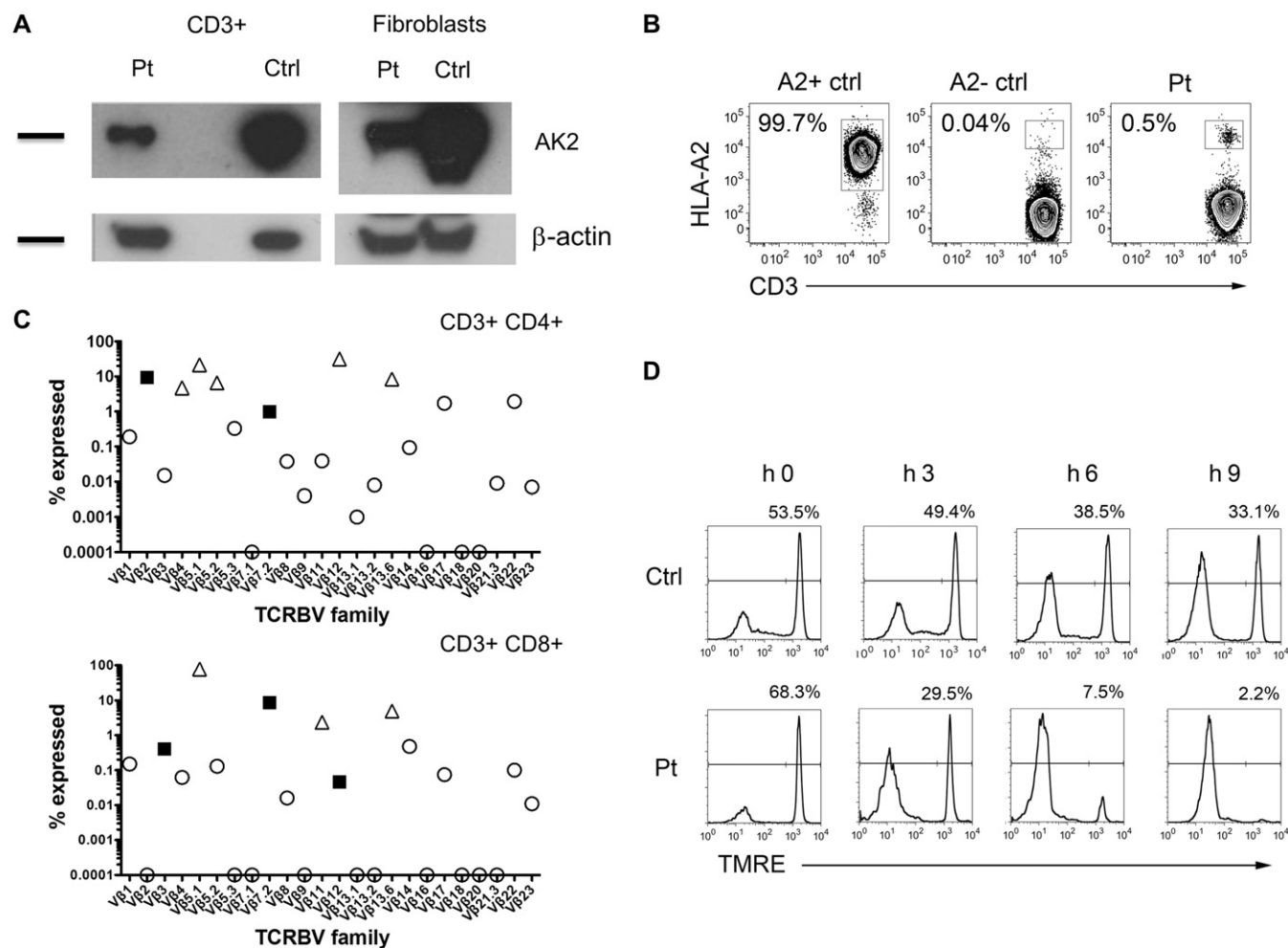


FIG 1. Characteristics of the infant's presentation. **A**, Expression of AK2 protein detected in patient's PBMC and fibroblasts (*Pt*) compared with normal control (*Ctrl*). Expression of β -actin is shown as a loading control. **B**, Maternal engraftment was excluded by flow analysis of the noninherited HLA-A2 allele. **C**, T-lymphocyte receptor oligoclonality was demonstrated by skewed expression of TCRV β families in the patient's CD3⁺CD4⁺ and CD3⁺CD8⁺ cells. Open circle, below normal; closed square, within normal range; open triangle, above normal. **D**, Disruption of mitochondrial membrane potential in CD3⁺ cells incubated with staurosporin and detected by using tetramethylrhodamine ethyl ester (TMRE) dye.

venous thrombosis and was treated with defibrotide. He subsequently developed progressive multiorgan dysfunction with fevers, respiratory insufficiency, hemodynamic instability, renal insufficiency, and coagulopathy. Similar to published cases of infants transplanted for immunodeficiency treated with corticosteroids,¹⁰ he developed cardiomyopathy with new severe concentric left ventricular hypertrophy. Cardiac biopsy on day +25 showed a mild T lymphocyte and histiocyte infiltrate and lack of myocyte hypertrophy. Rectal biopsy on day +28 demonstrated severe colitis consistent with acute graft versus host disease. Methylprednisolone was increased to 3 mg/kg per day, but despite this therapy, the patient died on day +31 after developing asystole. Postmortem examination was not performed.

We sought to confirm the diagnosis of RD by assessing AK2 protein expression and function. We also examined the etiology of this infant's rash, diarrhea, and lymphadenopathy in the setting of the expanded CD3⁺ T-lymphocyte population, considering maternal engraftment or OS as possible causes of the presentation.

AK2 protein was detected at reduced levels in the patient's CD3⁺ T cells and fibroblasts by Western blot (Fig 1, A). To determine whether this AK2 protein was derived from autologous or maternally engrafted T cells, we performed fluorescence in situ hybridization of peripheral blood cells (99% carried a Y chromosome), short tandem repeat analysis of purified CD3⁺ cells (0% maternal), and flow cytometry analysis (0.5% bearing the noninherited maternal HLA-A2 allele), thus ruling out maternal T-cell engraftment as the source of expanded T cells (Fig 1, B). Somatic reversion in T cells was ruled out by detecting the same AK2 mutation in purified CD3⁺ T lymphocytes (see Fig E2 in this article's Online Repository at www.jacionline.org). OS is characterized by the production and expansion of oligoclonal T cells, and indeed, the infant's CD3⁺, CD4⁺, and CD8⁺ T-cell receptor repertoire was highly oligoclonal, with only 5 of 24, 2 of 24, and 3 of 24 TCR β variable region families falling in the normal range, respectively (Fig 1, C). Purified CD3⁺ T lymphocytes showed susceptibility to apoptosis, evidenced by increased depolarization of the mitochondrial membrane potential at 3, 6, and 9

hours of incubation with staurosporin, indicating abnormal function of the mutant AK2 (Fig 1, D) (see additional information in this article's **Methods** section in the Online Repository at www.jacionline.org).

This infant's presentation of desquamative erythroderma, diarrhea, and generalized lymphadenopathy in the setting of an expanded and oligoclonal autologous CD3⁺ T-cell population is consistent with OS. This case indicates that at least some mis-sense AK2 mutations may result in residual T-lymphocyte development, oligoclonal expansion, and symptoms of OS. Thus, AK2 should be added to the list of severe combined immunodeficiency-causing genes that may manifest as OS.

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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).

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FIG E1. Patient exhibited desquamative erythroderma.

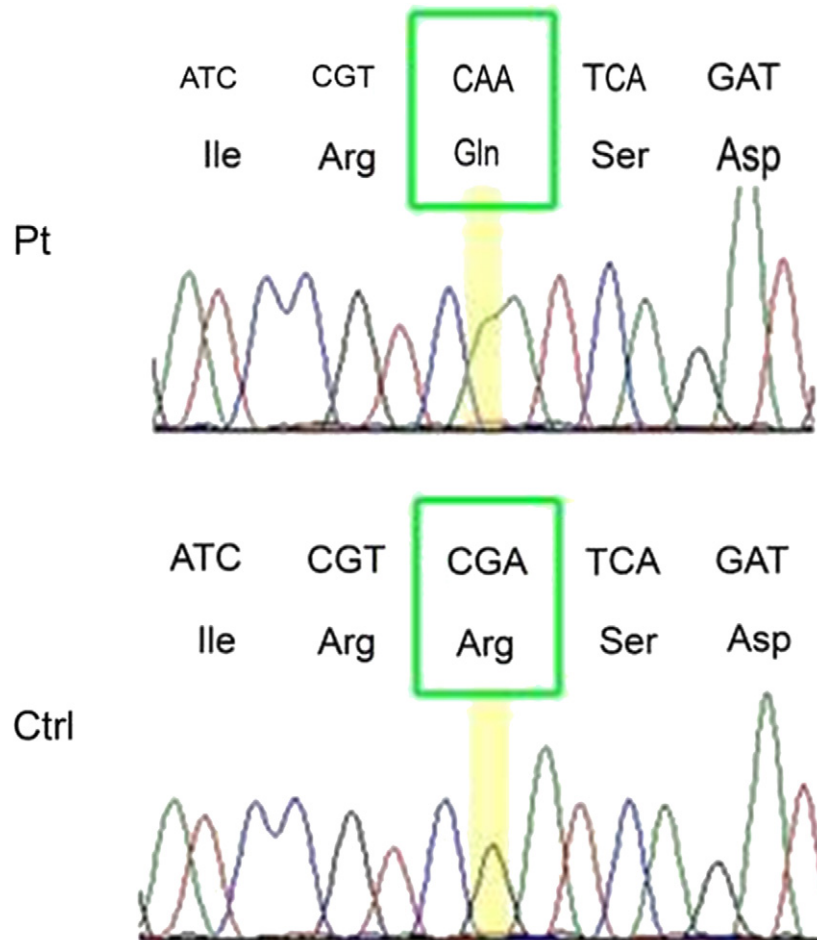


FIG E2. Analysis using genomic DNA from fibroblasts and CD3⁺ lymphocytes demonstrating a missense mutation in AK2 (c.524 G>A, p.R175Q).