

Clinical Communications

Effective and safe treatment of a novel IL2RA deficiency with rapamycin

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Clinical Implications

- We report an IL-2 receptor α chain deficiency caused by novel IL-2 receptor α chain compound heterozygous mutations. Rapamycin therapy effectively improved the clinical manifestations without any obvious side effects and may become a standard treatment for IL-2 receptor α chain deficiency.

TO THE EDITOR:

IL2RA (IL-2 receptor α chain) deficiency is a rare disease that presents with chronic infections and severe autoimmunity resembling Immunodysregulation Polyendocrinopathy Enteropathy X-linked (IPEX) syndrome. Until now, 11 cases from 9 unrelated families have been reported¹ (see Table E1 in this article's Online Repository at www.jaci-inpractice.org) and most of them manifest with severe autoimmune enteritis, bacterial, viral, and fungal infections, and pronounced lymphoproliferation.^{2,3} Because of different clinical manifestations, an effective and standard treatment has not been established for IL2RA-deficient patients. Rapamycin is considered to be an effective and safe therapeutic option for patients with IPEX and IPEX-like syndromes.^{4,5} Here, we report an IL2RA-deficient patient with a novel compound heterozygous *IL2RA* mutation and the efficacy and safety of rapamycin therapy.

A 9-year-old girl, born in 2010 to nonconsanguineous Chinese parents, presented with skin rashes, recurrent respiratory infection, and chronic diarrhea since the age of 2 months. Both her older sister and brother, born in 2003 and 2004, died at the age of 1 month because of diabetes mellitus, diarrhea, and pneumonia (Figure 1, A). It is unclear why 2 siblings succumbed at 1 month but could in part be attributable to the poor standard of medical care at the time. At age 8 years, the patient developed autoimmune hemolytic anemia with low hemoglobin levels (58 g/L) and positive Coomb test result. Antinuclear antibodies, perinuclear antineutrophil cytoplasmic antibodies, and antineutrophil cytoplasmic antibodies were also positive. She experienced autoimmune diabetes mellitus with polydipsia, polyphagia, polyuria, and nocturia, and her blood glucose level ranged from 11 to 22.4 mmol/L. She had diffuse lymphadenopathy, especially involving the cervical, subclavicular, and inguinal lymph nodes, and persistent chronic or recurrent splenomegaly and hepatomegaly. Moreover, she had aspergillus infection (Galactomannan test result in serum was 2.305,

reference < 0.5) and recurrent pneumonia. A computed tomography scan of her chest revealed a hilar and mediastinal nodular soft tissue mass shadow and bilateral bronchiectasis (Figure 1, D, left). She also showed growth retardation with 114 cm height at age 8 years (<2 SD). Serum IgA, IgG, IgM, and IgE levels were within the normal range. The proportions of CD3⁺ T lymphocytes and natural killer cells were decreased, whereas the percentage of B lymphocytes was increased. Both naive CD4⁺ and naive CD8⁺ T cells were reduced in the patient, whereas central memory (CM) CD4⁺ T cells and CM CD8⁺ T cells were increased. The proportion of naive B cells was increased, whereas proportions of memory B cells and transitional B cells were decreased (Table I).

Whole-exome sequencing revealed the presence of novel heterozygous compound mutations in the patient (Figure 1, B). The c.64G>A missense mutation from her mother is located right at the end of exon 1, which changed the conserved G nucleotide right before the GT splice donor site to A (Figure 1, C). TA cloning of patient's IL2RA cDNA and sequencing revealed that the actual splicing occurred right after c.60G (red arrow, Figure 1, C) instead of at c.64G for normal IL2RA cDNA (NM_000417.2), resulting in a frameshift mutation, p. Ala21Serfs*26 (see Figure E1 in this article's Online Repository at www.jaci-inpractice.org). The c.340C>T stop gain mutation from her father (Figure 1, B) located in exon 3 resulted in premature termination of protein translation, p. Gln114Ter (Figure 1, C, and see Figure E1, A). The patient's father and mother were heterozygous for the c.340C>T and c.64G>A mutation, respectively. Flow cytometry analysis confirmed the lack of CD25 (IL2RA) protein expression both on the cell surface (see Figure E2, A and B, in this article's Online Repository at www.jaci-inpractice.org) and in the cytoplasm (Figure E2, C) of patient's T cells (P) before and after anti-CD3 plus rhIL-2 or anti-CD28 stimulation, in contrast to T cells from her father (F), mother (M), or a healthy control (HC). RT-PCR analysis revealed that the heterozygous compound mutations did not affect *CD25* gene transcription (Figure E2, D).

The patient initially received treatments with anti-infection drugs, immunoglobulin replacement therapy, and prednisone; however, these treatments had no apparent effect on the symptoms. The parents of the patient refused hematopoietic stem cell transplantation. Considering her autoimmune symptoms, we started rapamycin therapy (1 mg/d) and simultaneously gradually reduced the prednisone dosage. The blood concentration of rapamycin was monitored and the trough concentration was maintained at 5 to 15 ng/mL. After rapamycin treatment for 1 month, the clinical manifestations had already improved in the patient, with a decrease in the severity and frequency of infections, diarrhea, skin rashes, and lymphadenopathy. The patient's pulmonary aspergilliosis also improved without any antifungal treatments (Figure 1, D). The size of the liver and spleen had clearly decreased. The symptoms of polydipsia, polyphagia, polyuria, and nocturia had disappeared after 3 months of rapamycin treatment, and her blood glucose returned to the normal range in the absence of any hypoglycemic agents. The antinuclear antibodies and Coomb test result turned

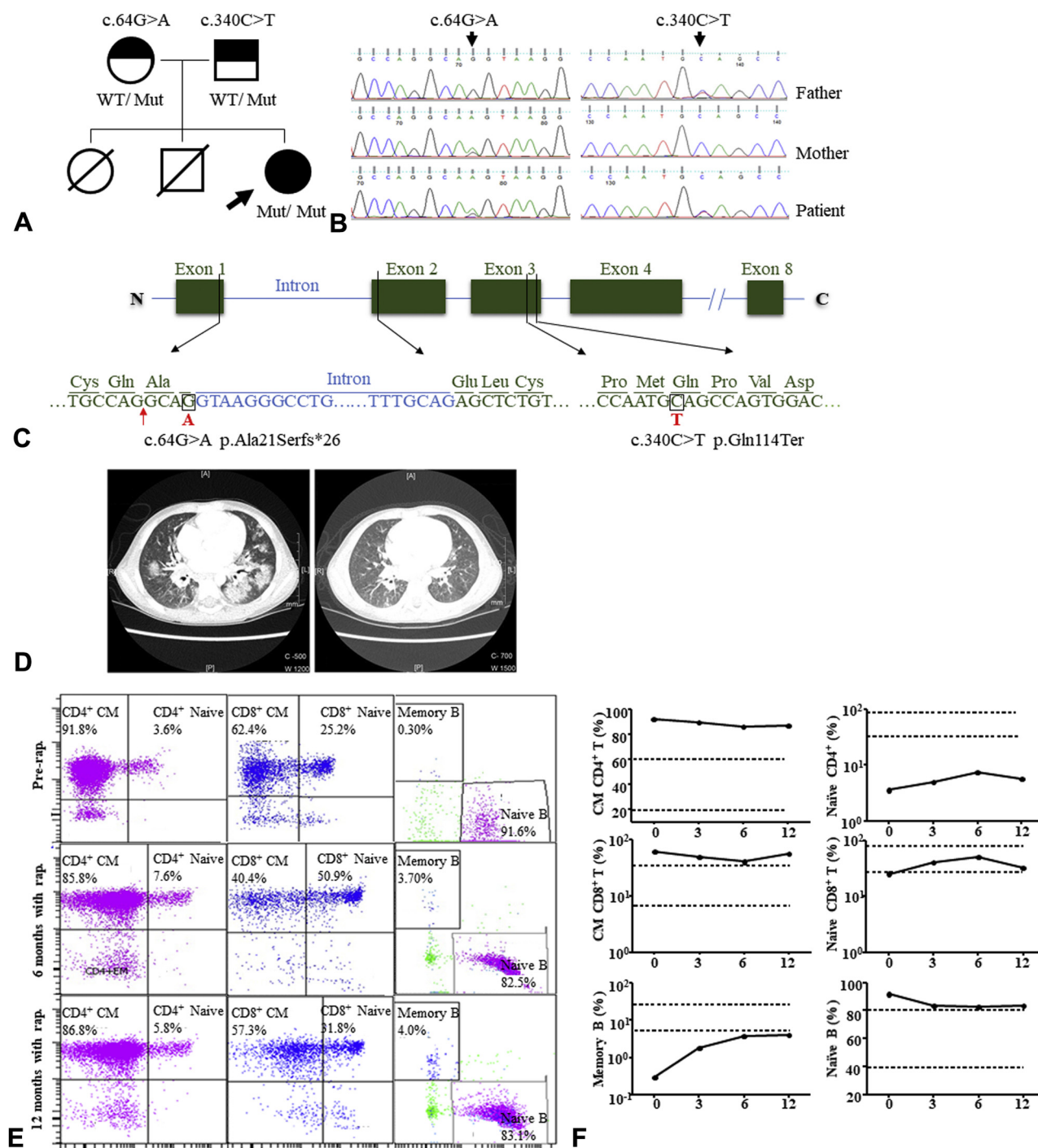


FIGURE 1. (A) Pedigrees of the patient (arrows). (B) Identification of mutation sites (arrows). (C) Mutated nucleotides are framed. A red arrow indicates new splice site. (D) Improved pulmonary aspergillosis after rapamycin treatment (right). (E) Representative fluorescence activated cell sorting profiles of patient's PBMCs. (F) Changes in patient's lymphocyte subsets during rapamycin therapy (dotted line, reference range of normal peers). Mut, Mutated; WT, wild-type.

negative, as did the anemia symptoms (Table I). Prednisone treatment was stopped after 6 months of rapamycin therapy. Consistent with the clinical improvements, her CD3⁺ T lymphocytes and natural killer cells had increased and CM CD4⁺ and CM CD8⁺ T cells had decreased, whereas naive CD4⁺ and

CD8⁺ T cells had increased, especially after the first 6 months of treatment. Memory and transitional B cells had increased, whereas naive B cells had decreased during rapamycin therapy (Figure 1, E and F, and Table I). Importantly, after prednisone treatment was stopped, the patient had been treated only with

TABLE I. Lymphocyte subsets changes and laboratory examinations of the patient during rapamycin therapy

Lymphocyte subsets %	0 mo	3 mo	6 mo	12 mo	Reference range
T cells (CD3 ⁺)	59.44*	62.7	73.37	66.61	64-73
T _H cells (CD3 ⁺ CD4 ⁺)	42.76*	53.1*	56.02*	49.22*	26-36
Naive T _H cells (CD4 ⁺ CD45RA ⁺ CD27 ⁺)	3.6*	4.9*	7.6*	5.8*	30.9-80.46
CM T _H cells (CD4 ⁺ CD45RA ⁻ CD27 ⁺)	91.8*	89.3*	85.8*	86.8*	17.37-60.96
Effector memory T _H cells (CD4 ⁺ CD45RA ⁻ CD27 ⁻)	4.6	5.7	6.5	7.2	1.18-13.30
Cytotoxic T cells (CD3 ⁺ CD8 ⁺)	15.52*	14.9*	13.55*	15.14*	24-34
Naive cytotoxic T cells (CD8 ⁺ CD45RA ⁺ CD27 ⁺)	25.2*	40.5	50.9	31.8	30.94-80.62
CM cytotoxic T cells (CD8 ⁺ CD45RA ⁻ CD27 ⁺)	62.4*	48.7*	40.4	57.3*	7.48-42.22
Effector memory cytotoxic T cells (CD8 ⁺ CD45RA ⁻ CD27 ⁻)	9.9	7	4.4	5.8	0.78-19.76
Terminal effector memory cytotoxic T cells (CD8 ⁺ CD45RA ⁺ CD27 ⁻)	2.4	3.7	4.3	5.1	0.4-30
Double-negative T cells (CD3 ⁺ CD4 ⁻ CD8 ⁻)	2.4	1.9	1.9	4.6	0.24-3.89
B cells (CD19 ⁺)	32.21*	16.1	12.8	15.32	9.19-19.48
Naive B cells (CD19 ⁺ IgD ⁺ CD27 ⁻)	91.6*	83.6*	82.5*	83.1*	38.94-79.48
Memory B cells (CD19 ⁺ IgD ⁻ CD27 ⁺)	0.3*	3.7*	1.8*	4*	5.7-25.4
Transitional B cells (CD19 ⁺ CD24 ⁺⁺ CD38 ⁺⁺)	0.1*	0.6*	1.9	3.4	1.66-16.52
Plasmablasts (CD19 ⁺ CD38 ⁺)	0.2*	2.1	1.1	1	0.5-18.52
NK cells					
NK cell (CD16 ⁺ CD56 ⁺)	5.32*	8.7	12.8	17.73	11-23
Lymphocyte numbers (cells/ μ L)					
T cells	885.8*	837*	736.5*	1200.7	1200-2480
T _H cells	637.04	597.16*	562.59*	887.28	621-1258
Cytotoxic T cells	231.29*	217.67*	136.11*	272.89*	509-1050
B cell	480.03	520.59	228.55	240.14	247-578
NK cells	79.35*	44.31*	108.55	319.67	203-584
Laboratory examinations					
Glucose (mmol/L)	17*	9*	4.3	4.2	3.89-6.1
Hemoglobin (g/L)	90*	118	125	117	>115
ANA	Positive	Negative	Negative	Negative	Negative
pANCA	Weakly positive	Negative	Negative	Negative	Negative
cANCA	Positive	Negative	Negative	Negative	Negative
Liver size [†]	5	4	3	2.5	0
Spleen size [‡]	5	2.3	1.5	0	0

ANA, Antinuclear antibodies; cANCA, antineutrophil cytoplasmic antibody; NK, natural killer; pANCA, perinuclear antineutrophil cytoplasmic antibody.

*Abnormal values.

[†]The sizes of liver were measured subxiphoid $\times$ cm.[‡]The sizes of spleen were measured infracostal $\times$ cm.

rapamycin without any other treatment. The patient has been followed up for 1.5 years after rapamycin treatment and now lives without any obvious symptoms, indicating that rapamycin therapy is highly effective and may become a standard treatment for IL2RA deficiency. Rapamycin had previously been used in the treatment of IL2RA-deficient patients and in support of our conclusion a partial rescue of T-cell phenotypes was observed^{4,5} (Table E1).

Because IL2RA plays a crucial role in regulating T-cell development and differentiation and in maintaining normal immune homeostasis,^{6,7} we analyzed the patient's T cells for any functional abnormalities. Her T cells showed decreased cell death after stimulation, a phenomenon known as activation-induced cell death,⁸ as compared with parents and HC (see Figure E3, A, in this article's Online Repository at www.jaci-inpractice.org). The patient's T cells appeared to be activated, judging from their increased cell sizes (Figure E3, B) and

elevated CD69 expression (Figure E3, C), but were unable to produce IFN- γ (Figure E3, D), in contrast to T cells from the parents and HC, which produced large amounts of the cytokine. We also measured IL-2 production and found that the culture supernatants of patient's T cells contained much higher levels of IL-2 (Figure E3, E), possibly due to the lack of CD25-mediated binding and consumption of the secreted IL-2.⁹ These results collectively suggest that the patient's T cells could only be partially activated and were thus resistant to cell death. We think that this T-cell phenotype is related to the seemingly conflicting immune deficiency and autoimmune phenotypes observed in this patient.

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ONLINE REPOSITORY

METHODS

This study was approved by the Ethics Committee of the Children's Hospital of Fudan University.

Patient

A female patient was diagnosed with IL2RA deficiency on the basis of her clinical manifestations, immunologic features, and genome sequencing. The patient's parents provided written informed consent for enrollment in this study.

Routine evaluation of immunologic function

Serum IgG, IgA, and IgM levels were determined by automated clinical chemistry analyzer (Erba, model: XL-200) as described previously.^{E1} IgG (catalog no. 67731), IgA (catalog no. 67746), and IgM (catalog no. 67732) reagents were all purchased from Orion Diagnostica Oy (Espoo, Finland). IgE was assessed by UniCAP (Pharmacia, Uppsala, Sweden). Staining for lymphocyte surface markers was performed after red blood cell lysis, according to a standard flow cytometric multicolor protocol with the appropriate fluorochrome-conjugated antibodies. After washing with cold PBS containing 2% FBS 2 times, 1×10^4 to 5×10^4 live cells were analyzed by FACSCalibur flow cytometer (Becton Dickinson, Franklin Lakes, NJ) using Diva software (BD Biosciences, Franklin Lakes, NJ). The following validated antibodies (BD Biosciences) were used for flow cytometry: anti-CD3 (UCHT1), anti-CD8 (RPA-T8), anti-CD27 (M-T271), anti-CD45RA (HI100), anti-CD4 (RPA-T4), anti-TCR $\alpha\beta$ (T10B9.1A-31), anti-TCR $\gamma\delta$ (B1), anti-CD19 (HIB19), anti-CD24 (ML5), anti-CD38 (HIT2), and anti-IgD (IA6-2).

Genetic analysis

Whole-exome sequencing and analysis protocols were adapted for genetic analysis. Briefly, genomic DNA samples were extracted from the whole blood of the patient and her parents. Then, genomic DNA fragments were enriched for the target region of the consensus coding sequence exons and subsequently sequenced on the HiSeq2000 sequencer (Illumina, San Diego, Calif). The raw data were mapped to the human genome reference sequence (hg19). Data analyses were performed in our clinical genetics laboratory by a bioinformatics team. Nucleotide changes observed in more than 5% of aligned reads were called and reviewed by using NextGENe software (SoftGenetics, State College, Pa).

Mutations in *IL2RA* were confirmed by using Sanger sequencing. Genomic DNA was extracted from the patient and parents. PBMCs were isolated using the RelaxGene Blood DNA System (Tiangen Biotech, Beijing, China) according to the manufacturer's protocol. Primers were designed to span each exon, and PCR amplification of *IL2RA* was carried out at 95°C for 5 minutes followed by 35 cycles of 95°C for 30 seconds, 60°C for 30 seconds, and 72°C for 40 seconds. Final extension was performed at 72°C for 7 minutes. The PCR product was purified by Performa DTR Gel Filtration Cartridges and sequenced in both directions by ABI Prism BigDye terminators.

TA cloning

The compound heterozygous mutations were identified using target gene capture combining with next-generation sequencing.

To confirm the mutation type, total RNA of patient's PBMCs was extracted and reverse transcribed using PrimeScript 1st strand cDNA synthesis kit (Takara, Dalian, China). CD25 cDNA fragment encompassing the 2 mutation sites was amplified by PCR using the high-fidelity KOD-plus DNA polymerase (Toyobo, Japan) and the forward (5'-CGGAATTCGG-CAGTTTCCTGGC-3') and reverse (5'-GCGTCGAC-GATTGTTCTTCTACTC-3') primers. Amplification was conducted at 94°C for 2 minutes followed by 32 cycles of 94°C for 15 seconds, 58°C for 30 seconds and 68°C for 70 seconds, and an extra extension at 68°C for 7 minutes. PCR products were purified with a PCR purification kit (GeneMark, Cat: DP034) and cloned into pMDTM20-T vector (Takara, Kyoto, Japan) according to the manufacturer's instructions. Nucleotide alterations were identified by sequence alignment with the CD25 GenBank sequence (NM_000417.2) using the BioEdit Sequence Alignment Editor software (Tom Hall, Carlsbad, Calif).

T-cell activation *in vitro*

PBMCs isolated from the patient, parents, and HC were washed and cultured at 37°C in RPMI 1640 containing 5×10^{-5} mol 2-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 0.1 mg/mL streptomycin, supplemented with 10% heat-inactivated FCS (all from ThermoFisher Scientific, Waltham, Mass). PBMCs (2×10^6 cells/mL) were stimulated in 24-well plates (Corning, NY) with plate-bound antihuman CD3 (2 μ g/mL, BD Biosciences) and antihuman CD28 (5 μ g/mL, BD Biosciences) or recombinant human IL-2 (10 ng/mL, BD Biosciences) at 37°C for 24 hours or with phorbol 12-myristate 13-acetate (5 μ g/mL, Sigma, Santa Clara, Calif) and ionomycin (Iono) (1 μ M, Sigma) for 4 hours. Activated cells were harvested for flow cytometry analysis or lysed with RNAiso plus (Takara) for RNA extraction. The supernatants were frozen for ELISA.

CD25 expression

For cell surface CD25 expression, PBMCs were washed with cold PBS containing 2% FBS and incubated with diluted antibodies at 4°C for 15 minutes in the dark. The following antibodies were used: fluorescein isothiocyanate-antihuman CD4 (BD Biosciences), phycoerythrin (PE)-Cy7 antihuman CD69 (BD Biosciences), PE-antihuman CD25 (R&D, Minneapolis, Minn; Immunogen: Glu22-Cys213), and 7-actinomycin D (BD Biosciences). The cells were then washed twice with cold PBS containing 2% FBS and resuspended in 0.3 mL of PBS containing 2% FBS. For CD25 cytoplasmic expression, PBMCs were first stained with fluorescein isothiocyanate-antihuman CD4 to label T cells and antihuman CD25 (Basiliximab) (Novus Biologicals, Minneapolis, Minn; immunogen: Glu116-Val122) to block cell surface CD25. After washing, the cells were fixed and permeabilized with perm/fix buffer (BD Biosciences) and stained with PE-antihuman CD25 (R&D, Immunogen: Glu22-Cys213) at room temperature for 30 minutes in the dark. The cells were then washed with perm buffer, resuspended in 0.3 mL of PBS containing 2% FBS, and 100,000 7-actinomycin D⁻ CD4⁺ cells were analyzed with a FACSVerse flow cytometer using the FACSuite software (BD Biosciences). Fresh PBMCs were used as a control.

RT-PCR

To determine CD25 mRNA expression under various activation conditions, total RNA was extracted and reverse transcribed using PrimeScript 1st strand cDNA synthesis kit (Takara). The cDNAs were amplified with 2×Es Taq MasterMix (Dye) (CWBIO) using CD25 forward (5'-GTGGTGGGGCAGATGGTTTA-3') and reverse (5'-TTGTGACGAGGCAGGAAGTC-3') primers or β -actin forward (5'-AGCGAGCATCCCCAAAGTT-3') and reverse (5'-GGGCACGAAGGCTCATCATT-3') primers. Amplification was conducted in 20 μ L solution at 94°C for 2 minutes, followed by 32 cycles of 94°C for 15 seconds and 60°C for 30 seconds. An extra extension at 72°C was performed for 10 minutes. Samples were resolved on a 1.5% agarose gel and visualized by staining with ethidium bromide. Full function of gel image analysis system FR-1000 was used for analysis.

Cytokine detection

IL-2 and IFN- γ cytokine production was analyzed by ELISA (Thermo Fisher Scientific) according to the manufacturer's instructions. All samples were assayed in duplicates.

Statistical analysis

Data obtained from flow cytometry were analyzed with FlowJo 7.6 Software (Treestar, Woodburn, OR), exported to Excel spreadsheets, and subsequently analyzed with GraphPad Prism 6 (San Diego, Calif). Results are presented as mean $\pm$ SD. Statistical analysis of the data was performed with Welch *t* test (**P* < .05, ***P* < .01, ****P* < 0.001); values of *P* less than .05 were considered statistically significant.

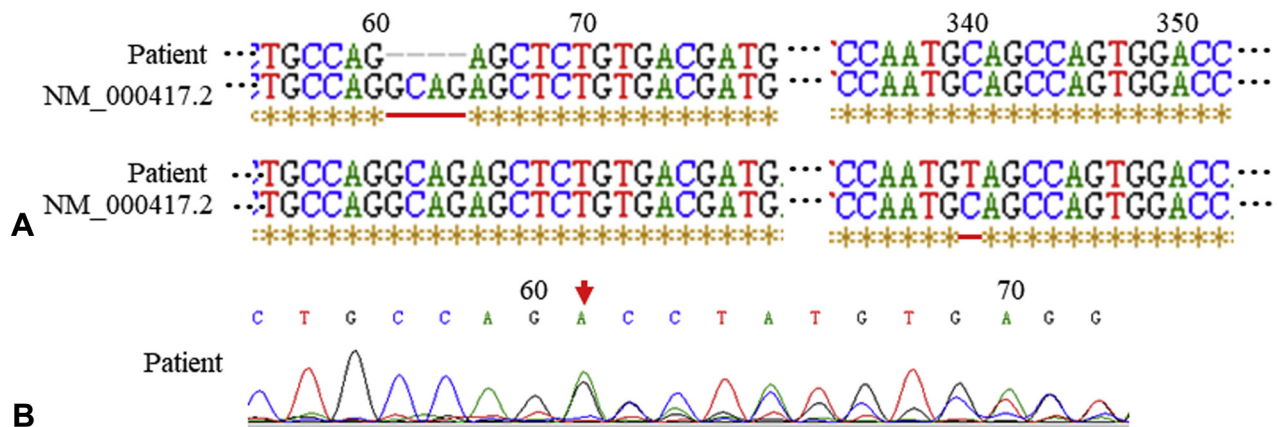


FIGURE E1. (A) CD25 cDNA sequence of the patient has a 4-bp deletion (61-64) and a c.340C>T substitution (both underlined in red). (B) Direct sequencing of the PCR product of patient's CD25 cDNA revealed chaotic peaks from 61-bp (indicated by a red arrow).

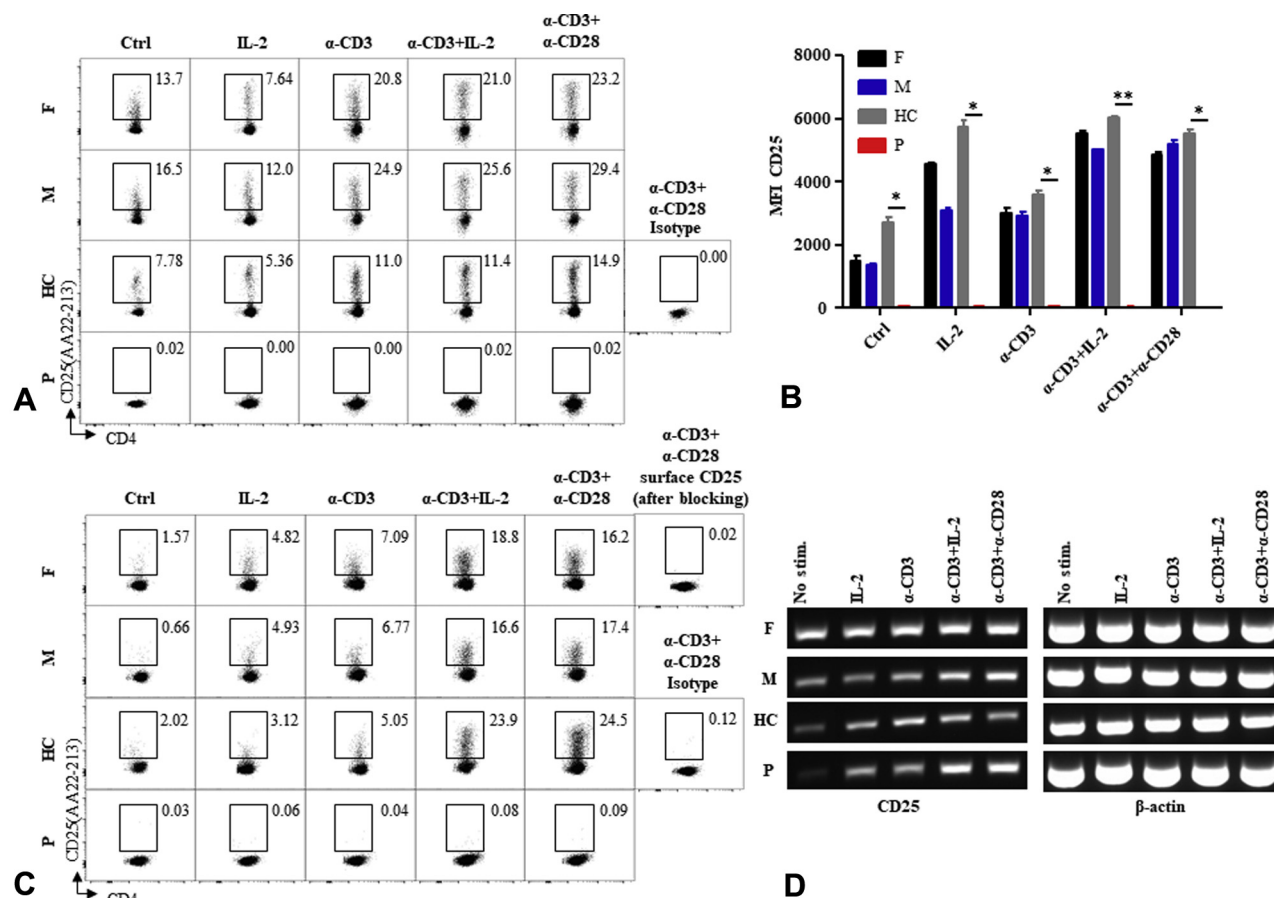


FIGURE E2. (A) CD25 surface expression in cultured T cells. (B) MFI of cell surface CD25 in gated CD4⁺CD25⁺ T cells shown in Figure E2, A. (C) Intracellular CD25 expression in cultured T cells. (D) CD25 mRNA expression in cultured T cells. All experiments were independently repeated twice. MFI, Mean fluorescence intensity. * $P < .05$. ** $P < .01$.

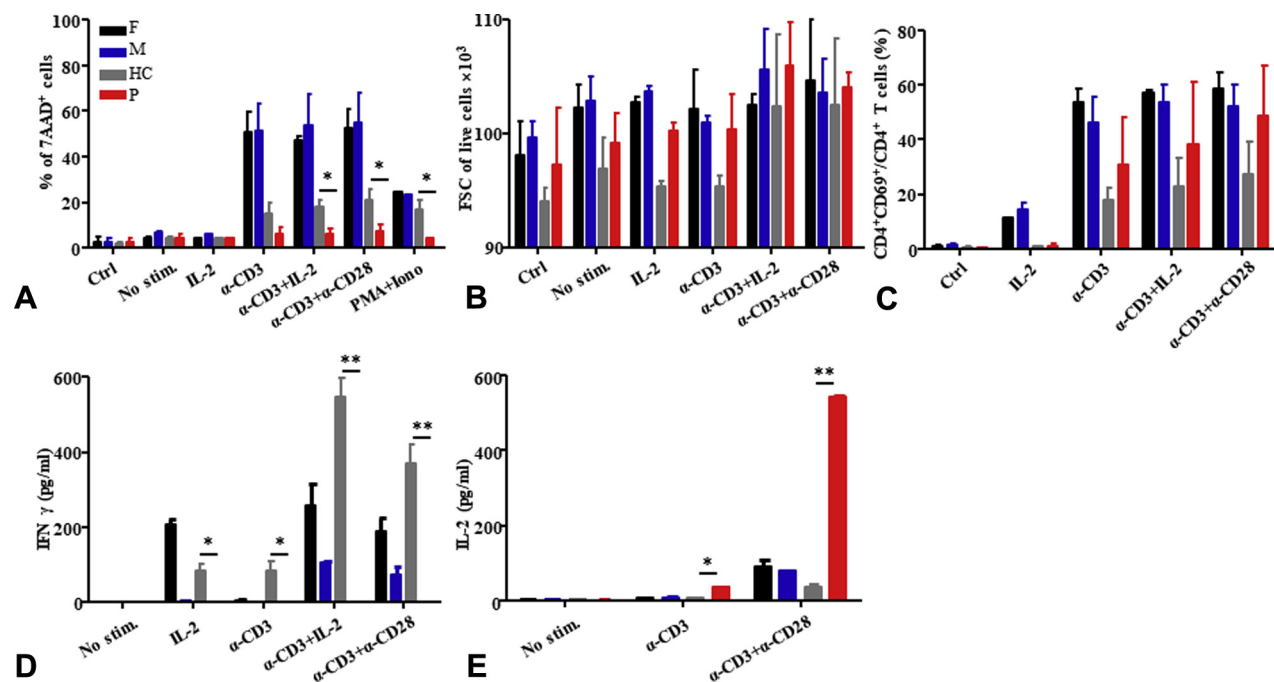


FIGURE E3. (A) Proportion of 7AAD⁺ dead cells. (B) FSC (cell size) of live lymphocytes. (C) CD69⁺ cells among CD4⁺ T cells. (D and E) The amount of IFN- γ and IL-2 in culture supernatants, respectively. Ctrl, Control; Stim., stimulation; 7AAD, 7-actinomycin D. Ctrl, fresh PBMC; no stim., PBMCs cultured in medium alone. * $P < .05$. ** $P < .01$.

TABLE E1. Summary of reported IL2RA-deficient patients

Reference	Sex, age	Therapy	Outcome	Response to rapamycin treatment
E2	Male	An allogeneic bone marrow transplant after cytoreduction	A complete resolution of symptoms	NA
E3	Male, 8 y	Corticosteroids, rituximab, intravenous immunoglobulin, cyclosporin A, and antibiotics	NA	NA
E4	Female, 8 y	Steroids, tacrolimus, mycophenolate mophetil, and rapamycin	NA	Improvement of the lesions
E5	Female, 5 y	Oxygen therapy, immunosuppressive treatment with corticosteroids, antibiotic prophylaxis, rapamycin, and intravenous gammaglobulin	NA	Improved condition, and no abnormalities in hematological cell counts, liver enzymes, cholesterol levels, or renal function
E6	Male, infant	Methylprednisolone, rituximab, cyclophosphamide, mycophenolate mofetil	Improvement of lung disease, cessation of bleeding; blood cell counts and bilirubin levels normalized	NA
E7	Male, 17 y	Intravenous immunoglobulin and prophylaxis with azithromycin	Reduced infections	NA
E8	Female, 12 y	Methylprednisolone, cyclosporin A, intravenous injections, and bone marrow transplantation	NA	NA
E9	1 Female 2 Males	Immunosuppressive therapy	NA	NA
E10	Male, 2 mo	Underwent HSCT from matched unrelated donor	A complete resolution of the symptoms	NA
Current report	Female, 9 y	Anti-infection drugs, prednisone (stopped after 6 mo), rapamycin	The clinical manifestations had significantly improved	The patient has been followed up for 1.5 y and lives without any obvious symptoms

HSCT, Hematopoietic stem cell transplantation; NA, not available.

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