

Granulomatous hepatitis in a Saudi child with *IL2RA* defect: a case report and literature review

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Abstract: Interleukin-2 receptor alpha (*IL2RA*) defect (OMIM- # 606367) is an immune disease where affected patients are vulnerable to developing recurrent microbial infections in addition to lymphadenopathy and dermatological manifestations. This condition is known to be caused by pathogenic variants in the *IL2RA* gene, which are inherited in an autosomal recessive fashion. In this case report, we present a patient with *IL2RA* defect from Saudi Arabia who presented with chronic diarrhea, poor weight gain, mild villous atrophy, malnutrition, hepatomegaly, nonspecific inflammation, and an eczematous skin rash. His genetic analysis revealed a novel, homozygous, and likely pathogenic variant, that is, c.504 C>A (Cys168Ter), located in the exon 4 of the *IL2RA* gene, which was inherited from his parents in an autosomal recessive mode of inheritance. This variant produces a 272-amino-acid shorter *IL2RA* protein chain, which most likely becomes degraded in the cytosol. Thus, we assume that the c.504 C>A is a null allele that abolishes the synthesis of *IL2RA*, malforms the IL-2 receptor complex, and eventually causes immunodeficiency manifestations. To our knowledge, this is the first time a person with *IL2RA* defect has shown signs of granulomatous hepatitis on a liver biopsy.

Keywords: CD25, granuloma, hepatitis, *IL2RA*, immune disease, immunodeficiency

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Introduction

Interleukin receptor 2 alpha (*IL2RA*) defect (OMIM- # 606367) is an autosomal recessive disorder of immune system dysregulation caused by homozygous or compound heterozygous mutations in the *IL2RA* gene, located on chromosome 10p15. Hyperproliferation of CD8⁺ T cells occurs owing to defects in T-cell regulation, weak antibody response, and increased production of cytokines, as demonstrated by immunology investigations.¹ This condition usually presents in infancy with recurrent infections, lymphadenopathy, autoimmune enteropathy, and eczematous dermatitis.¹ Here we describe a case with an *IL2RA* mutation that had elevated liver enzymes with a liver biopsy showing granulomatous hepatitis.

Case report

A 12-year-old Saudi Arabian boy of consanguineous parents first presented in 2009 at the age of 7 months with chronic diarrhea, poor weight gain, malnutrition, and an eczematous skin rash. Anti-tissue transglutaminase IgA was slightly elevated [39.3 units, upper limit of normal (ULN) = 0–20] and mucosal biopsy of the duodenum showed mild villous atrophy and nonspecific inflammation [Figure 1(c)]. The patient failed to gain weight normally following a trial of a gluten-free diet, despite partial improvement of diarrhea on lactose-free and amino acid formulas. Skin manifestations were progressive, involving the whole skin surface with areas of localized dermatitis and scaling [Figure 1(a) and (b)]. Skin biopsy revealed mild hyperkeratosis with foci of lymphocytic

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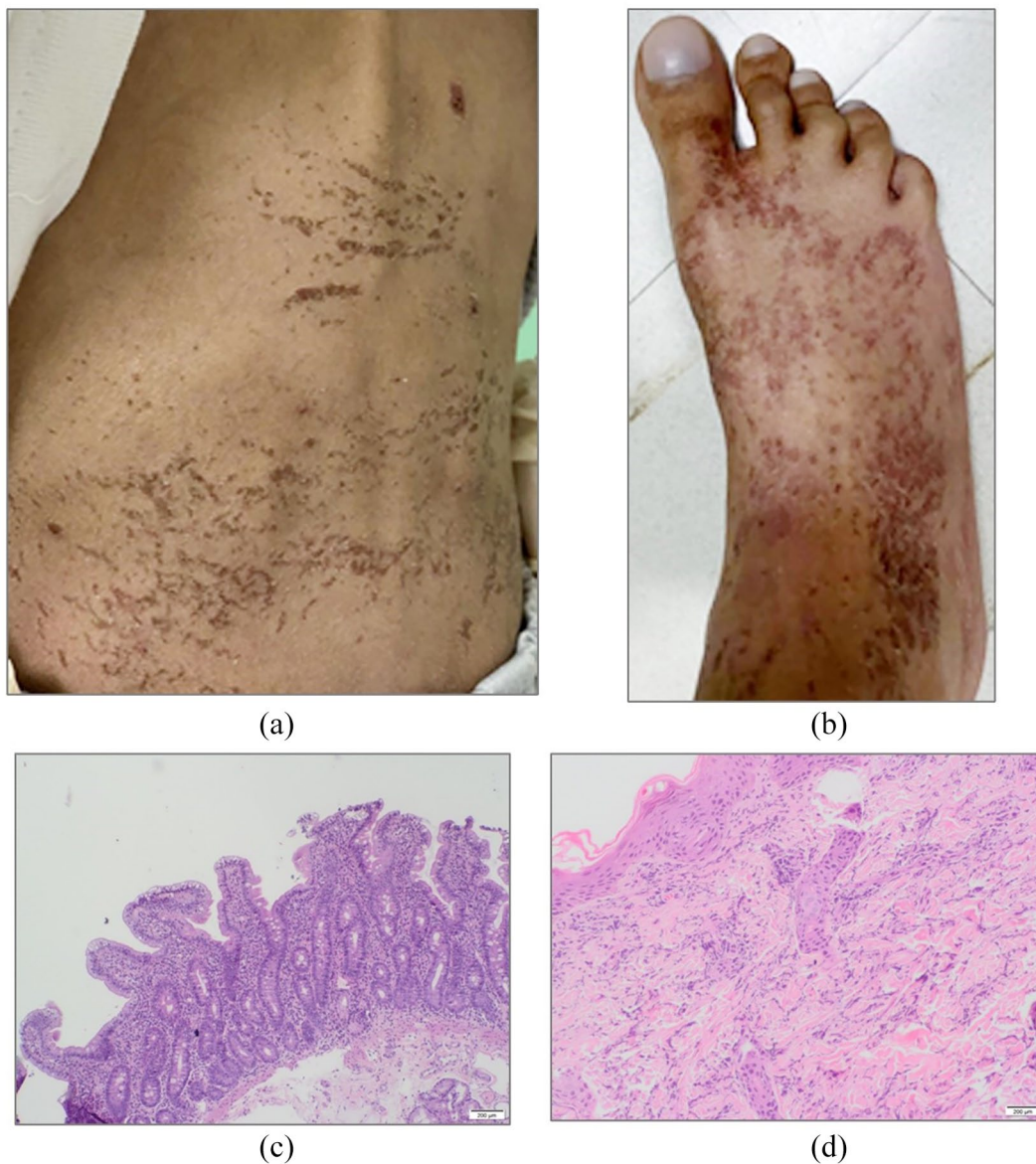


Figure 1. (a) and (b) Area of localized scaly dermatitis and clubbing. (c) Histological findings in the duodenum. Low power view revealing moderate villous shortening and intraepithelial lymphocytosis. (Hematoxylin & Eosin stain, 5 \times). (d) Dermatitis. Skin biopsy showed superficial and deep moderate mononuclear infiltrate (Hematoxylin & Eosin stain, 10 \times).

exocytosis and interstitial chronic inflammatory infiltrate, mainly consisting of lymphocytes, with perivascular and peri-appendiceal aggregation seen mainly in the superficial and deep layers of the dermis [Figure 1(d)]. There was one episode of urinary tract infection with *Klebsiella pneumoniae* organism, which was treated with antibiotics. Renal ultrasonography showed bilateral miniscule renal stones that were detected on serial imaging studies.

At the age of 2 years, hepatomegaly with a moderate increase in liver enzymes was found (γ -glutamyl transferase = 211 U/l, aspartate amino transferase = 132 U/l, alanine amino transferase = 226 U/l, alkaline phosphatase = 382 U/l, bilirubin = 9 μ mol/l). Percutaneous liver biopsy was performed, which showed expansion of the portal tracts and distension with chronic inflammatory cells, mainly lymphocytes and occasionally eosinophils, along with an ill-defined

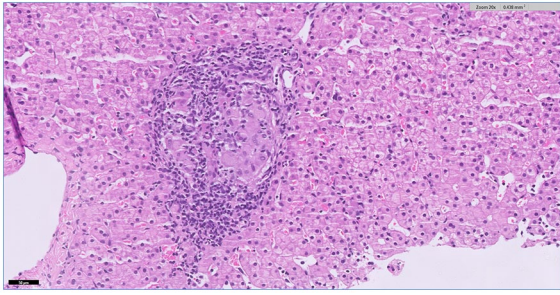


Figure 2. Granulomatous hepatitis. Some of the portal tracts contain ill-defined noncaseating granulomas formed of epithelioid histiocytes that are surrounded by dense mononuclear infiltrate (Hematoxylin & Eosin stain, 20×).

granuloma surrounded by dense inflammatory cells (Figure 2). Screening for autoimmune markers, including anti-smooth muscle antibody (SMA), anti-nuclear antibody (ANA), and anti-liver kidney microsomal antibody-1 (LKM-1), was negative as were the immunoglobulin (Ig) levels [IgG 19.5 g/l (5.4–16.1 g/l), IgA 3.87 g/l (0.8–2.8 g/l), IgM 1.35 g/l (0.5–1.9), and IgE 77 kU/l (0–195 kU/l)]. Liver enzymes declined with time, although liver enlargement continued.

At 4 years of age, following adenotonsillectomy, the boy developed mild splenomegaly and generalized lymphadenopathy of the cervical, axillary, and inguinal lymph nodes, accompanied by digital clubbing. Test results for tuberculosis (tuberculin skin test and chest X-ray), sarcoidosis, and parasitic infections were negative.

At 9 years of age, owing to persistent diarrhea, failure to grow, and a high fecal calprotectin level (6200 mg/kg), upper gastrointestinal endoscopy and colonoscopy were performed. Pancolitis was found during colonoscopy and normal upper gastrointestinal endoscopy. Chronic active inflammation was reported in the biopsy specimens, taken from the esophagus, stomach, duodenum, and colonic mucosa, with marked lymphocytic infiltration. In consideration of a possible diagnosis of IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked) syndrome, a screening for possible endocrinopathies was performed, but the thyroid function tests with thyroid antibodies were negative, and there were normal fasting blood sugar and hemoglobin A1c levels. There was no evidence of autoimmune neutropenia, anemia, or thrombocytopenia.

The patient continued to have alternating diarrhea and normal bowel motions. Anthropometric measures for his age were always under the third percentile for weight, height, and body mass index. Unfortunately, the patient passed away aged 10 years after developing septic shock.

Written informed consent was obtained from the parents for genetic testing and for reporting the case. Under HIPAA (Health Insurance Portability and Accountability Act), ethical approval is not needed for case reports.

Family pedigree and molecular studies

Figure 3 shows the family pedigree of the patient being studied. The whole exome sequencing of the proband was performed on the Illumina, HiSeq2000 sequencing platform at 30× coverage. The full details of DNA purification, library preparation, sequencing, data filtration, and variation identification strategies we adopted are outlined in our recent publications.^{2–4} From the exome data of the proband, we observed a novel, homozygous, and likely pathogenic c.504 C>A variant located in the exon 4 region of the *IL2RA* gene, resulting in the substitution of a cysteine codon at amino acid position 168 with a termination codon in the *IL2RA* protein. Sanger sequencing of the proband and of both parents has confirmed the autosomal recessive mode of inheritance of this variant. This variant is absent from the GnomAD, 1000 Genomes Project, Saudi Human Genome Program, and DALIA databases. The functional characterization of the *IL2RA* variant was performed by various systems biology methods. First, the 3D model of *IL2RA* variant protein was constructed using the *ab-initio* method as described.⁵ Then, RNAfold webserver was used to estimate the effect of the *IL2RA*, c.504 C>A variant on the corresponding RNA structure.^{6,7} Findings from mRNA secondary structure of *IL2RA* predictions have indicated no significant changes in the thermodynamic stability of the mRNA folding pattern due to c.504 C>A variant (Figure 4). Finally, the NCBI conserved domain database was used to map the *IL2RA*, c.504 C>A variant on the functional domains of the protein molecule.⁸ From the results of functional domain analysis, we identified that the c.504 C>A variant falls into a canonical sushi-like functional domain (complement control protein, CCP), spanning between the 125th and 184th amino acids, and it most likely

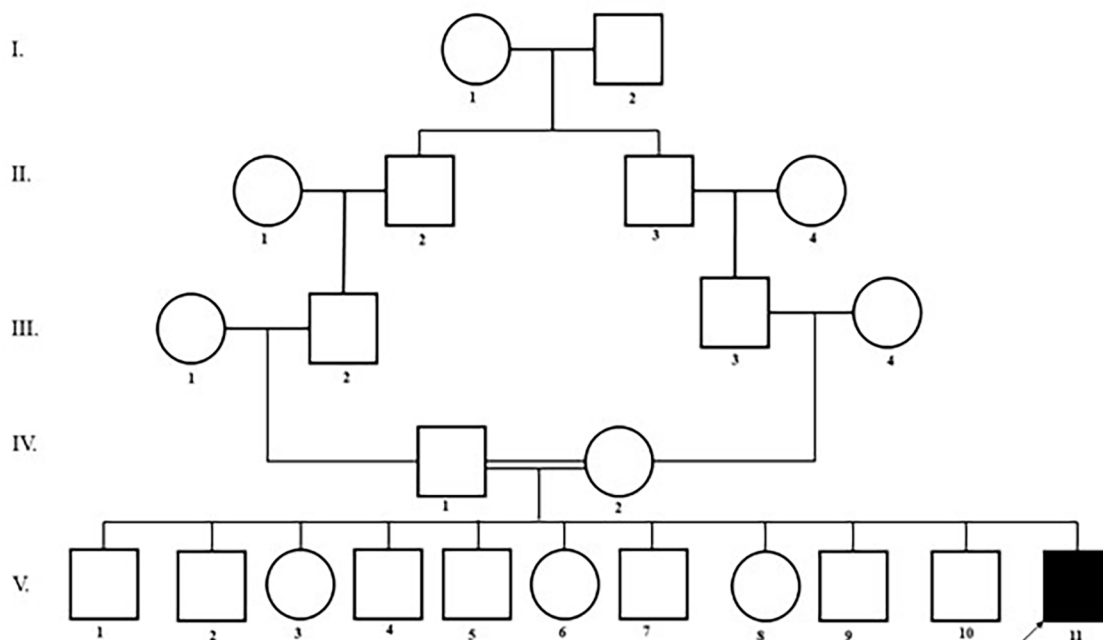


Figure 3. A multigeneration family pedigree showing the autosomal recessive mode of inheritance of *IL2RA* defect in the patient.

produces a premature protein (104 amino acids) that is shorter than the native *IL2RA* protein molecule (272 amino acids).

Discussion

Human IL-2 receptor α chain deficiency is an immune dysregulation disorder involving dysfunctional interleukin-2 (IL-2) receptors and occurs owing to mutations in the α chain (CD25).⁹ The IL-2 binds to IL-2R (IL-2 receptor), which is basically composed of three subunits, namely, α (CD25), β (CD122), and γ (CD132). The β and γ chains are predominantly located on T lymphocytes, whereas α chain is mainly expressed on activated mature T lymphocytes during early thymocyte development stages.¹⁰ All three subunits (α , β , and γ chains) are required for a functional IL-2 receptor.¹ The CD25 is constantly expressed by CD4+CD25+FOXP3+regulatory T cells, which allows them to be the initial responders to IL-2 in an immune response.¹¹ It also enhances IL-2 signaling by stimulating the FOXP3 transcription.¹² Single nucleotide polymorphism in *IL-2A* has been associated with multiple autoimmune disorders, such as type 1 diabetes.¹³ Upon antigenic stimulation, CD25 is essential for the

expansion of effector T cells in response to IL-2.¹ Goudy *et al.*¹ have suggested that IL-2 consumption by FOXP3+ Tregs is an important step in maintaining immune system homeostasis. The absence of CD25 on the surface of FOXP3+ Tregs, however, helps CD8+ T cells grow and causes autoimmune reactions in CD25-deficient patients.

Tregs is divided into thymus-derived (tTregs) and induced Tregs (iTregs). The tTregs develop in the thymus, but iTregs differentiate from naïve T-cell precursors peripherally. The generation in the thymus depends on the presentation of thymic self-peptide/MHC activation, which activates the precursors to differentiate into functional antigen-specific suppressive regulatory T cells. This process needs the activation of a number of Treg-specific enhancers, CD25, Ctl4, Eos, and Helios, which become gradually activated at the precursor stage before FoxP3 expression. The differentiation of peripheral iTregs, however, most likely occurs in response to nonself antigens, for example, commensal microbiota, food, and allergens, and depends on the signaling strength and abundance of the antigen, giving harmony of immune tolerance.¹⁰ Treg generation is largely dependent

on the presence of IL-2, required for homeostatic maintenance for their thymic development.¹¹ IL-2 is produced mainly by CD4+ T cells and cannot be synthesized by Treg cells. IL-2 has also been shown to be crucial for the development and maintenance of Treg cells. IL-2 also directly affects suppressive function of Tregs.¹²

One study has found that mice with IL-2 or IL-2 receptor deficiency display an enlargement of peripheral lymphoid organs, including lymph nodes and spleen, impaired activation-induced cell death, and autoimmune disorders, which have been attributed to a decreased generation of Tregs.¹³

IL2RA, along with two other receptors, *IL2RB* and *IL2RG*, forms a complex cell-surface receptor for the pleiotropic cytokine IL-2. When IL-2 binds to the receptor, the Jak3 (tyrosine kinase) and STAT5 (signal transducer) 7 proteins are activated.¹⁴ The *IL2RA* structure is characterized by D1 and D2 domains linked by linker peptides (T⁶⁵ to H¹⁰³), and the second domain has a 54-amino-acid-residue-long peptide, which connects it to the cell membrane (G¹⁶⁵ to E²¹⁷). It is thought that deleterious genetic mutations can eliminate or considerably reduce gene function. This could be true in the present case, since the c.504 C>A nonsense mutation introduces a protein termination codon at the 168th codon and truncates the *IL2RA* 104 amino acids shorter than the original *IL2RA*. The mRNAs with protein truncating code (PTC) often undergo nonsense-mediated decay¹⁵ or generate premature proteins, which will eventually undergo a ubiquitin-mediated protein degradation pathway.¹⁶ Thus, we assume that the c.504 C>A is a null allele that abolishes the synthesis of *IL2RA* and malforms the IL2 receptor complex. Because IL-2R is not available on the surface of resting T and B cells, but expressed by aberrant T cells in patients with leukemia, autoimmune conditions, or organ transplant rejection, it is considered to be an ideal target for therapeutic modulation.^{17,18} In recent years, some molecular studies have shown that the effect of nonsense mutations can be mitigated in the *LDLR* gene by using translation inhibitory molecules, such as aminoglycoside antibiotics, which were shown to be effective in partial restoration of full-size proteins encoded by genes with premature stop mutations.¹⁵ A recent study has shown that pathogenic mutations

identified in the *IL2RA* gene (CD25) in siblings presenting monogenic autoimmunity can be corrected in their affected primary T cells using the CRISPR-Cas9 genome-targeting system.¹⁹ Whether a similar strategy could be useful to treat patients harboring *IL2RA* mutations, however, has not yet been established.

A few case reports have been published regarding *IL2RA* defect patients.^{1,9,20,21} Sharfe *et al.* describe a novel case of an *IL2RA*-deficient patient who suffered from chronic infections and severe autoimmunity, resembling immune dysregulation, polyendocrinopathy, enteropathy, and X-linked (IPEX) syndrome, caused by mutations in the *FOXP3* gene. This IPEX-like patient possessed a translation frameshift mutation in the *IL2RA* gene, abating its expression. Similarly, a second report²¹ describes a patient with a different frame shift mutation in the *IL2RA* gene, leading to a CD25 null phenotype with comparable clinical manifestations. A third report by Goudy *et al.* involved a patient with the development of progressive manifestations of both autoimmunity, such as enteropathy, erythroderma, and severe alopecia, and immunodeficiency with chronic cytomegalovirus (CMV) infection. A case study reported by Bezrodink *et al.* involved a girl with CD25 mutation-associated chronic inflammatory lung disease (follicular bronchiolitis with lymphocytic hyperplasia).²⁰

To our knowledge, the present case report is the first instance in the medical literature where an immunodeficiency-41 patient has shown positive evidence of developing granulomatous hepatitis on liver biopsy. The patient developed hepatitis and had extensive examination that excluded autoimmune and infectious causes, including CMV.

A liver biopsy revealed portal tract expansion and distension with chronic inflammatory cells, primarily lymphocytes and occasionally eosinophils, as well as an ill-defined granuloma surrounded by dense inflammatory cells. The examination rules out other causes of granulomatous liver disease, including sarcoidosis, tuberculosis, Crohn's disease, and fungal infections. Liver enzymes, however, improved gradually with no specific treatment, although there was ongoing hepatomegaly. No further elevation of liver enzymes was found. A recent study has provided preclinical

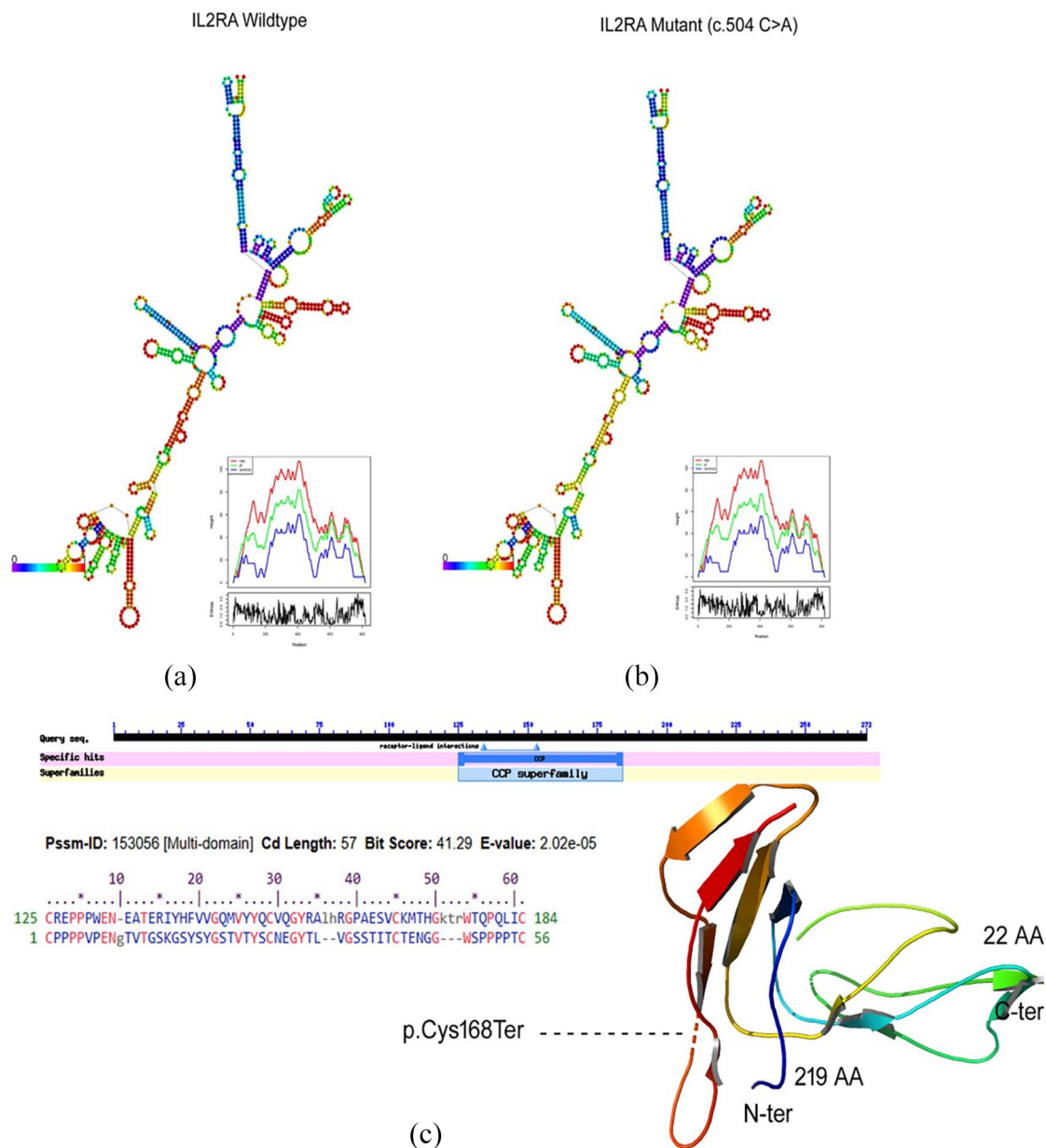


Figure 4. Bioinformatic analysis of the human *IL2RA*. Images (a) and (b) show the RNA secondary structure predictions for wild-type and mutant mRNAs (c.504 C>A) in the form of a mountain plot (MP) representation of minimum free energy (MFE), thermodynamic ensemble (pf), and the centroid structure predictions, respectively. The color gradient in the scale of 0 to 2 represents the MFE calculations of nucleotide base pairing. The MP representation shows the secondary structures in a height *versus* position in which the helices are represented in slopes, loops in plateaus, and hairpin loops in the peaks. The bottom graph represents the entropy of predicted RNA structure in which higher entropy means the RNA structure has lower stability. Image (c) shows that the c.504 C>A variant falls in the complement control protein (CCP domain). Also, the location of Cys168Ter is shown in the 3D model.

evidence that CRISPR-Cas9-based genome targeting has the potential to correct the pathogenic *IL2RA* variant in primary T cells from a monogenic autoimmune disease family.¹⁹ Thus, we

presume that the *IL2RA* variant identified in our patient can also be targeted by nonviral genome-targeting methods in primary T cells *in vitro* or *in vivo* in order to pave the way for future

personalized medicine in patients suffering with the IL2RA defect.

Declarations

Ethics approval and consent to participate

Under HIPAA (Health Insurance Portability and Accountability Act), ethical approval is not needed for case reports. Written informed consent was obtained from the parents for genetic testing and for reporting the case.

Consent for publication

Written informed consent was obtained from the parents of the patient for publication of details of the case report. Personal information was not mentioned in a way that can lead to the identification of the patient or family.

Author contributions

Meshari A. Alaifan: Data curation; Formal analysis; Methodology; Writing – review & editing.

Ohood Abusharifah: Conceptualization; Methodology; Resources; Writing – original draft.

Rana Yagoub Bokhary: Resources; Visualization; Writing – review & editing.

Babajan Banaganapalli: Data curation; Formal analysis; Investigation; Project administration; Resources; Writing – review & editing.

Noor Ahmad Shaik: Data curation; Investigation; Resources; Visualization; Writing – review & editing.

Naglaa M. Kamal: Writing – review & editing.

Omar I. Saadah: Conceptualization; Data curation; Formal analysis; Methodology; Resources; Software; Supervision; Writing – original draft; Writing – review & editing.

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Competing interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

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