International Journal of Immunopathology & Pharmacology

Case Report

International Journal of Immunopathology and Pharmacology
Volume 35: 1–5

DOI: 10.1177/20587384211025934
journals.sagepub.com/home/iji
SAGE

Sirolimus is effective in autoimmune lymphoproliferative syndrome-type III: A pedigree case report with homozygous variation PRKCD

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Abstract

Autoimmune lymphoproliferative syndrome (ALPS) usually presents in childhood with fever, nonmalignant splenomegaly, and lymphadenopathy along with cytopenia, which is caused by mutations in the FAS apoptotic pathway. The TCR$\alpha\beta$ + CD4/CD8 double-negative T cells (DNT), one of required criteria of ALPS, will rise markedly in ALPS. Human Protein kinase C delta (PRKCD) deficiency (OMIM # 615559) was recently identified to be causative for an ALPS-type III with significant B-cell proliferation particularly of immature B cells. We report a pedigree homozygous variation of PRKCD gene (c.36T>G, p. Y12X) which presented with refractory cytopenia, splenomegaly, and polarization of DNT/regulatory T cells (Treg) axis. After repeated recurrence, the patient was treated with mTOR inhibitor sirolimus, which had a safety mechanism and specifically rebalance the DNT/Treg axis. The patient's hemoglobin and clinical condition improved gradually by the application of sirolimus (1.5 mg/m^2, actual blood concentration 4.27–10.3 ng/l). Homozygous variation in PRKCD may lead to typical ALPS clinical manifestations. Targeting DNT/Treg axis, use of sirolimus in such patients may help to achieve good clinical control.

Keywords

autoimmune lymphoproliferative syndrome-type III, DNT, PRKCD variation, Treg

Date received: 18 October 2020; accepted: 27 May 2021

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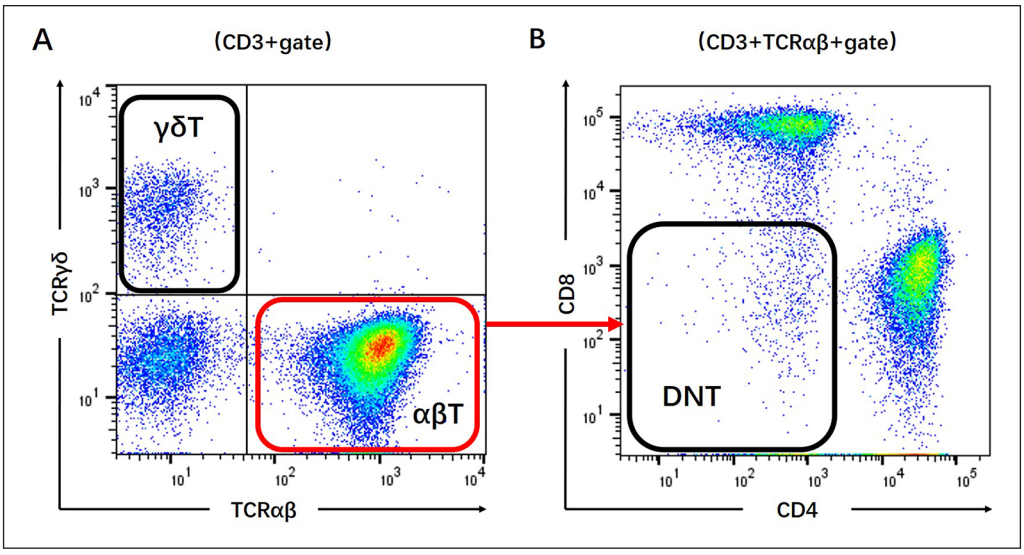

Figure 1. The portion of TCRαβ + CD4 / CD8 double-negative T cell touch the diagnose criteria of ALPS.[5]

Introduction

ALPS is due to defective function of the Fas death receptor, which results in defective apoptosis of activated lymphocytes, and often involves autoimmune manifestations.[1,2] Deleterious heterozygous mutations in the FAS gene are the most common cause of failed lymphocyte apoptosis, termed ALPS-FAS.[1,2] DNTs, which may accumulate with either somatic or germ-line FAS mutations and constitutively express IL-10 are important mediators of disease.[3] ALPS-FAS is most frequently caused by heterozygous mutations that generate mutant FAS proteins, often with defective death domains.[4] Over the past years, although improvements in genomic technologies have led to the description of a number of ALPS-like autoimmune and lymphoproliferative disorders, ALPS-like is currently unknown in a considerable percentage of patients. Another difficulty when dealing with ALPS-like patients is that subjects with autoimmune cytopenias, required corticosteroid-sparing therapy like sirolimus. These patients were recently categorized as having ALPS-related syndrome (ARS).[5]

Protein kinase C delta (PRKCD) is activated via diacylglycerol produced by receptor-mediated hydrolysis of membrane inositol phospholipids as well as by phorbol ester.[6] Numerous studies in humans and mice have established that variation on PRKCD may affect B-cell signaling and autoimmunity, as well as regulation of growth, apoptosis, and differentiation of a variety of cell types.[7–10] Interestingly, clinical manifestations of the so far reported cases exhibit some of the signs of the ALPS-like disease, including autoimmunity, cytopenia, and increased B-cell numbers and proliferation. In this pedigree report, we describe the clinical and laboratory findings of homozygous PRKCD variant, presenting features of ALPS symptom with raised ratio of DNTs and a low percentage of Treg and Th17.

Case report

Patient presentation

This article presents a case study of a patient with homozygous variation of PRKCD and clinical manifestation of ALPS, especial elevation of TCRαβ + CD4/CD8 double-negative T cells (DNTs) (Figure 1). The age of disease onset and diagnosis was 6 and 10 years old respectively. From 2015, he showed unexplained large-scale splenomegaly and lymphadenopathy, along with recurrent infection. Severe neutropenia and anemia presented in July 2017. The patient experienced pancytopenia and was refractory to first-line treatment of immune thrombocytopenia like steroids (HD-Dex, use 0.6 mg/(kg·days) for 4 days every 4 weeks), immunoglobulins (800 mg/kg). The positive phenotype of DNTs were 2.15% of $CD3^+$ and 1.64% for lymphocyte. His immunoglobulin levels were within normal range, elevated for ESR and positive of immunoglobulin G for anti-Epstein Barr viruses, rubella viruses, cytomegalovirus, herpes simplex virus, and antinuclear antibodies. In addition, the analysis of markers for measles virus, parvovirus B19, schistosomiasis, and Leishmania donovani infection was

Table 1. Basic immunologic data.

	Initial (2017.7)	Post-sirolimus (2018.3)	Stop-sirolimus (2019.1)	Normal ranges
$CD3^+$T cells (% of lymphocytes)	81.9	63.37	78.74	50.0–84.0
$CD4^+$T cells (% of T cells)	38	47.15	45.34	30.0–67.0
$CD8^+$T cells (% of T cells)	60.8	39.32	54.18	23.0–50.0
$CD19^+$ (% of lymphocytes)	9	18.5	16.6	9.19–19.48
$CD16^+56^+$ (% of lymphocytes)	3.9	9.8	6.8	10.01–26.98
$CD3^+4^+25^+Foxp3^+$T cells (% of $CD4^+$T cells)	3	8.59	2.98	4.10–9.40
DNT (% of $CD3^+$T cells)	2.15	1.77	2.4	<2.5
DNT (% of lymphocytes)	1.64	1.12	1.97	<1.5

negative. In peripheral blood samples, T lymphocytes ($CD3^+CD19^-$) were found to be increased. Moreover, the patient displayed amplified proportions of cytokine IFN-γ, IL-10, IL-6, however, the serum level of the vitamin B12 is normal. Histological analysis of the bone marrow and spleen did not show signs of hemophagocytosis and Lymphoma.

Based on glucocorticoids, sirolimus (1.5 mg/m^2, actual blood concentration range: 4.27–10.3 ng/ml) was added as the other immunosuppressive agent, after which platelet count was restored and spleen recovered to its normal size gradually in 3 months. However, unauthorized withdrawal of sirolimus for half a year, all clinical manifestations relapsed.

The study has been approval by the Medical Ethics Committee of Beijing Children's Hospital (2018-k-97) patient consent. The patient has provided informed consent for publication of the case.

Laboratory findings

Routine blood results indicated anemia (hemoglobin 7.8–9.0 g/dl) and thrombocytopenia (56–73 $\times$ 10^9/l). Three months after application of sirolimus, platelets (160 $\times$ 10^9/l) and hemoglobin (10.5 g/dl) were raised. The patient displayed persisting T-cell lymphocytosis with a decreased proportion of T-helper lymphocyte ($CD3^+CD4^+$), especially Treg ($CD3^+4^+25^+Foxp3^+$T cells). Reduction of DNT and increase of Treg portion was observed after treatment of sirolimus. The percentage of lymphocyte subpopulation, especial DNT and Treg, back to initial condition after withdrawal of sirolimus (Table 1)

Genetic findings

We use whole-exome sequencing to screen for suspicious mutations and a homozygous nonsense variation (c.36T>G, p. Y12X) was detected on the PRKCD gene, then we use sanger sequencing for verification on PRKCD gene. After receiving informed consent, we sequenced parents and their daughter who perform both anemia and splenomegaly and lymphadenopathy but not as severe as the boy. The results show that parents are Heterozygote while the boy's sister is homozygous as well. It was verified as a consanguineous marriage of the parents. The variant was not found in dbSNP database or ExAC database (Figure 2).

Clinical course

Medical history, clinical presentation, and results from laboratory tests led to the clinical diagnosis of ALPS-related syndrome, and treatment with sirolimus (1.5 mg/m^2, blood concentration 5–15 ng/l) was initiated. Subsequently, the patient's hemoglobin and clinical condition improved gradually. Then, the relapsed clinical manifestations and imbalance DNT/Treg axis may be related to withdrawal of sirolimus for half year.

Discussion

In the present case report, we describe the phenotype of a pedigree presenting with splenomegaly, hepatomegaly and recurrent cytopenia. We identified a homozygous nonsense variation (c.36T>G, p. Y12X) in PRKCD. Similar to typical ALPS patients, abnormal DNT/Treg axis which can be rebalance by mTOR signal pathway inhibitor sirolimus[8–10] was obvious in this patient. DNT, in vitro apoptosis assay and sFASL level are optimal biomarker for typical ALPS, especially DNT for all type of ALPS.[10] Although only few patients were identified so far with PRKCD deficiency, they show a significant heterogeneity with regard to clinical manifestations. Recently, Sharifinejad et al. reported a 13-year-old boy, a homozygous frameshift mutation (c.1293_1294insA) in exon 13 of the PRKCD

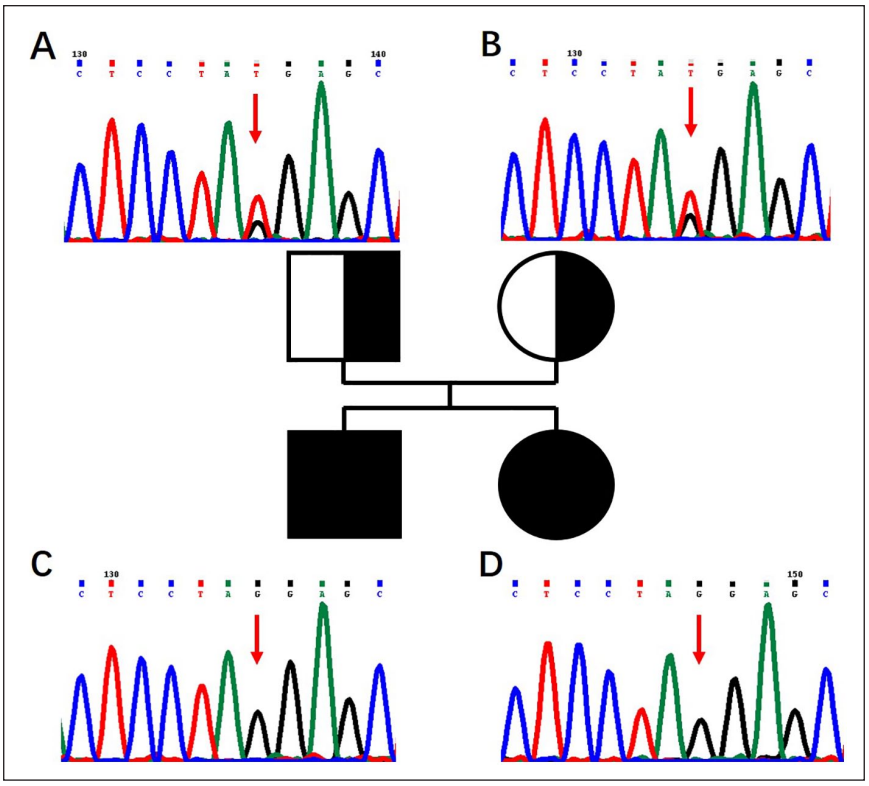

Figure 2. Pedigree of patient. Parents admit that they are intermarriage. The patient and his sister are homozygous for this change.

gene who presented with autoimmunity, lymphoproliferation, recurrent pneumonia, cardiomyopathy, and dermatological manifestations. An elevation of double-negative T cells, $CD8^+$ T cells, serum IgG level, as well as a reduction in NK cells, was observed in the patient.[7] In the literature review of the three PRKCD patients, all of them show an elevated TCRαβ + CD4/CD8 double-negative T cells (DNT). The early clinical onset of the disease and prominent autoimmunity in PRKCD deficiency was demonstrated to be associated with an abnormal B cell compartment, characterized by increased naive and diminished memory cells as well as high number of TCRαβ + CD4/CD8 double-negative T cells (DNT). Although T cell functions were reported to be preserved in previous patients, we detected abnormal DNT and Treg lymphocyte subpopulation in our case. Persistent infection may be the result of defective function and/or low numbers of NK cells and T helper cells. This patient showed a dermatological manifestations on the face at the onset of disease which improved after the topical application of antifungal drugs.

It is worth noting that the application of sirolimus in this patient established a favorable improvement in clinical symptoms. This improvement was associated with a decrease in DNT, which showed dose-dependence of sirolimus through the withdrawal event. This population of cells is reported to be increased in Common Variable Immune Deficient (CVID) patients with autoimmunity and splenomegaly, particularly as a required criteria for ALPS,[5] and has also been observed previously for PRKCD deficiency.[6,7] However, treatment with sirolimus in PRKCD disorders had not been previously described, even the effect of sirolimus on PRKCD has not yet been fully elucidated, it is known to have immunomodulatory, anti-inflammatory, anti-proliferative, and photoprotective effects.[11] Recent data on the sirolimus mechanism revealed an immunomodulatory effect through inhibition of mTORC1 in $CD4^+$, $CD8^+$, DNT, and Teff ($CD4+CD25^{low}$) cells thereby inducing rebalance of Treg, mTORC1 low expression, of these cells.[12] Various reports showed that sirolimus inhibits proliferative responses to T-cell mitogens and alloantigens and reduce some pro-inflammatory cytokines such as interleukin (IL)-6 and tumor necrosis factor alpha.[13] In addition, the successful use of sirolimus for management of other monogenic PID such as LRBA deficiency was also reported.[14] In summary, this case of children with PRKCD gene variation leading to ALPS-type III progress of the typical

clinical phenotype, including rapid progression of spleen lymphadenopathy, relapsed anemia, and polarization DNT/Treg axis. The remission and dose dependence due to rapamycin makes us concern the fluctuations of mTOR related T cell subpopulations.

Conclusion

Homozygous variation in PRKCD may lead to typical ALPS clinical manifestations. Targeting DNT/Treg axis, use of sirolimus in such patients may help to achieve good clinical control.

Declaration of conflicting interests

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

Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Ethical approval to report this case was obtained from Ethics Committee of Beijing Children's Hospital, Capital Medical University (2018-k-97).

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported in part by grants from Beijing Natural Science Foundation of China (No. 7192046), the Pediatric Medical Coordinated Development Center of Beijing Municipal Administration of Hospitals (No. XTZD20180205), National Science and Technology Key Projects (No. 2017ZX09304029004), Beijing Municipal Administration of Hospitals Clinical Medicine Development of Special Funding (code ZY201404) Support.

Informed consent

Written informed consent was obtained from the patient(s) for their anonymized information to be published in this article.

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