Original Article

Abatacept as a Long-Term Targeted Therapy for LRBA Deficiency

Ayca Kiykim, MD[a], Ismail Ogulur, PhD[a], Esra Dursun, MD[a], Louis Marie Charbonnier, PhD[b], Ercan Nain, MD[a], Sukru Cekic, MD[c], Dilek Dogruel, MD[d], Neslihan Edeer Karaca, MD[e], Mujde Tuba Cogurlu, MD[f], Ozlem Arman Bilir, MD[g], Murat Cansever, MD[h], Hasan Kapakli, MD[i], Dilek Baser, MSc[a], Nurhan Kasap, MD[a], Seyhan Kutlug, MD[j], Derya Ufuk Altintas, MD[d], Ahmad Al-Shaibi, MSc[k], Nourhen Agrebi, PhD[k], Manolya Kara, MD[l], Ayla Guven, MD[m], Ayper Somer, MD[l], Cigdem Aydogmus, MD[n], Nuray Aktay Ayaz, MD[o], Ayse Metin, MD[p], Metin Aydogan, MD[q], Aysen Uncuoglu, MD[q], Turkan Patiroglu, MD[h], Alisan Yildiran, MD[j], Sukru Nail Guner, MD[i], Sevgi Keles, MD[i], Ismail Reisli, MD[i], Guzide Aksu, MD[e], Necil Kutukculer, MD[e], Sara S. Kilic, MD[r], Mustafa Yilmaz, MD[d], Elif Karakoc-Aydiner, MD[a,s], Bernice Lo, PhD[k], Ahmet Ozen, MD[a,s], Talal A. Chatila, MD, MSc[b], and Safa Baris, MD[a,s]

Istanbul, Bursa, Adana, Izmir, Kocaeli, Ankara, Kayseri, Konya, and Samsun, Turkey; Boston, Mass; and Doha, Qatar

What is already known about this topic? LPS-responsive beige-like anchor deficiency presents with susceptibility to infections, autoimmunity, and lymphoproliferation. Abatacept treatment can control immune dysregulatory disease manifestations.

What does this article add to our knowledge? Long-term treatment with abatacept is effective in controlling disease activity. Superior clinical responses are achieved with a weekly or biweekly drug-dosing regimen. Lymphoproliferation and chronic diarrhea demonstrated the best responses to abatacept therapy, followed by other immune dysregulatory manifestations. The circulating T follicular helper cells are a reliable biomarker for monitoring disease activity.

How does this study impact current management guidelines? The results of this study may be helpful in the management, follow-up, and prediction of the response rate to abatacept as a tailored therapy for LPS-responsive beige-like anchor deficiency.

BACKGROUND: LPS-responsive beige-like anchor (LRBA) deficiency presents with susceptibility to infections, autoimmunity, and lymphoproliferation. The long-term efficacy of cytotoxic T-lymphocyte–associated antigen 4-immunoglobulin (abatacept) as targeted therapy for its immune dysregulatory features remains to be established.

[a]Division of Pediatric Allergy and Immunology, Marmara University School of Medicine, Istanbul, Turkey
[b]Division of Immunology, Boston Children's Hospital and Department of Pediatrics, Harvard Medical School, Boston, Mass
[c]Division of Pediatric Allergy and Immunology, Faculty of Medicine, Uludag University, Bursa, Turkey
[d]Division of Pediatric Allergy-Immunology, Faculty of Medicine, Çukurova University, Adana, Turkey
[e]Division of Pediatric Allergy and Immunology, Faculty of Medicine, Ege University, Izmir, Turkey
[f]Division of Pediatric Allergy and Immunology, Faculty of Medicine, Kocaeli University, Kocaeli, Turkey
[g]Division of Pediatric Hematology, Ankara Children's Hematology Oncology Training and Research Hospital, Ankara, Turkey
[h]Division of Pediatric Immunology, Faculty of Medicine, Erciyes University, Kayseri, Turkey
[i]Division of Pediatric Allergy and Immunology, Faculty of Medicine, Necmettin Erbakan University, Konya, Turkey
[j]Division of Pediatric Allergy and Immunology, Faculty of Medicine, Ondokuz Mayis University, Samsun, Turkey
[k]Division of Translational Medicine, Research Branch, Sidra Medicine, Doha, Qatar
[l]Division of Pediatric Infectious Diseases, Istanbul Medical Faculty, Istanbul University, Istanbul, Turkey
[m]Division of Pediatric Endocrinology Clinic, Medical Faculty, Zeynep Kamil Women and Children Hospital, Saglik Bilimleri University, Istanbul, Turkey
[n]Division of Pediatric Allergy and Immunology, Kanuni Sultan Suleyman Training Hospital, Istanbul, Turkey
[o]Division of Pediatric Rheumatology, Kanuni Sultan Suleyman Training Hospital, Istanbul, Turkey
[p]Division of Pediatric Immunology, Ankara Children's Hematology Oncology Training and Research Hospital, Ankara, Turkey
[q]Division of Pediatric Gastroenterology, Faculty of Medicine, Kocaeli University, Kocaeli, Turkey
[r]Division of Pediatric Immunology and Rheumatology, Faculty of Medicine, Uludag University, Bursa, Turkey
[s]Istanbul Jeffrey Modell Diagnostic and Research Center for Primary Immunodeficiencies, Istanbul, Turkey

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Corresponding author: Safa Baris, MD, Division of Pediatric Allergy/Immunology, Marmara University School of Medicine, Fevzi Çakmak Mah. No. 41, Istanbul, Turkey 34668. E-mail: safabaris@hotmail.com and sbaris@marmara.edu.tr.

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Abbreviations used
CD- Chronic diarrhea
CR- Complete remission
cT_{FH}- Circulating T follicular helper
CTLA4- Cytotoxic T-lymphocyte–associated antigen 4
DM- Diabetes mellitus
HSCT- Hematopoietic stem cell transplantation
ID- Immune dysregulation
LP- Lymphoproliferation
LRBA- LPS-responsive beige-like anchor
MFI- Mean fluorescence intensity
NK- Natural killer
PR- Partial remission

OBJECTIVE: To determine the clinical and immunologic features of LRBA deficiency and long-term efficacy of abatacept treatment in controlling the different disease manifestations.
METHODS: Twenty-two LRBA-deficient patients were recruited from different immunology centers and followed prospectively. Eighteen patients on abatacept were evaluated every 3 months for long-term clinical and immunologic responses. LRBA expression, lymphocyte subpopulations, and circulating T follicular helper cells were determined by flow cytometry.
RESULTS: The mean age of the patients was 13.4 ± 7.9 years, and the follow-up period was 3.4 ± 2.3 years. Recurrent infections (n = 19 [86.4%]), immune dysregulation (n = 18 [81.8%]), and lymphoproliferation (n = 16 [72.7%]) were common clinical features. The long-term benefits of abatacept in 16 patients were demonstrated by complete control of lymphoproliferation and chronic diarrhea followed by immune dysregulation, most notably autoimmune cytopenias. Weekly or every other week administration of abatacept gave better disease control compared with every 4 weeks. There were no serious side effects related to the abatacept therapy. Circulating T follicular helper cell frequencies were found to be a reliable biomarker of disease activity, which decreased on abatacept therapy in most subjects. However, high circulating T follicular helper cell frequencies persisted in 2 patients who had a more severe disease phenotype that was relatively resistant to abatacept therapy.
CONCLUSIONS: Long-term abatacept therapy is effective in most patients with LRBA deficiency. © **2019 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2019;■:■-■)**

Key words: *LPS-responsive beige-like anchor; Immune dysregulation; Abatacept; T follicular helper cells; Autoimmunity*

INTRODUCTION

LPS-responsive beige-like anchor (LRBA) deficiency is a primary immunodeficiency characterized by recurrent sinopulmonary infections with hypogammaglobulinemia, lymphoproliferation (LP), and immunodysregulation, which presents by enteropathy, cytopenias, and autoimmune endocrinopathy. LRBA plays a pivotal role in the intracellular trafficking of cytotoxic T-lymphocyte protein-4 by rerouting it away from lysosomal degradation and back to the cell surface.[1,2] Cytotoxic T-lymphocyte protein-4 is a key immune checkpoint protein that is constitutively expressed on fork-head box $P3^{+}$ regulatory T cells and is also induced on activation of conventional T cells.[3] LRBA deficiency results in very low cytotoxic T-lymphocyte–associated antigen 4 (CTLA4) expression, which explains the phenotypic overlap between LRBA- and CTLA4-deficient subjects.[4,5] Furthermore, reduced regulatory T-cell number and function have been demonstrated in LRBA-deficient patients.[6,7] Consequent upon this, LRBA deficiency may manifest as an immune dysregulation, polyendocrinopathy, enteropathy, and X-linked–like disease with early onset autoimmunity.[8,9]

LRBA was originally described as a common variable immunodeficiency–like disease with autoimmunity.[10,11] Two longitudinal cohorts were subsequently published that dwelt on the clinical and immunologic features of LRBA deficiency.[7,12] To date, different agents have been applied in the treatment of LRBA deficiency, including corticosteroids, intravenous immunoglobulin therapy, sirolimus, infliximab, rituximab, and azathioprine.[7,13] Some patients also benefit from hematopoietic stem cell transplantation (HSCT), which can be curative.[14] More recently, studies have suggested the effectiveness of abatacept, a CTLA4-immunoglobulin fusion protein, in controlling disease-related immune dysregulatory phenotypes.[1,13] In addition, some biomarkers such as soluble CD25 and circulating T follicular helper (cT_{FH}) cells were described as useful to monitor patients' disease activity.[15] Nevertheless, the long-term effectiveness of abatacept is not well documented. Also, there is no established consensus as to the dose and frequency of abatacept therapy for the treatment of LRBA deficiency and which biomarker is most reliable for follow-up of patients. The spectrum of the clinical responses of LRBA-deficient patients to abatacept treatment is also obscure.

In this report, we present the findings on a well-defined LRBA-deficient cohort, in which we prospectively evaluated the clinical and immunologic responses to abatacept therapy. Our studies establish the efficacy of long-term abatacept therapy in curbing the immune dysregulatory features of this disease in most cases and also highlight the limitations of this therapy in occasional patients with severe disease phenotype.

METHODS

Patient and inclusion criteria

The study included 22 patients with proven *LRBA* mutation. The patients were recruited from 12 different pediatric immunology centers in Turkey. They were enrolled into the study at different time points starting from November 2016 and followed up prospectively until December 2018. The study protocol was approved by the local ethics committee of Marmara University (institutional review board no. IRB00009067), and a written informed consent was obtained from all parents. Because of the young age of our patients, a simple oral description of the study was presented to participating children in the presence of their parent(s) and a verbal assent was requested.

Study design

The LRBA-deficient patients were enrolled to the study prospectively from related centers. During the study, baseline demographic, clinical, and immunologic data were collected. Blood samples from all the participating patients from the respective medical centers were sent to the Marmara University Pediatric Allergy and Immunology laboratory for immunologic assessment, including extensive lymphocyte subset analysis, cT_{FH} cell enumeration, and intracellular LRBA and CTLA4 staining. The changes in lymphocyte subsets and cT_{FH} cells were evaluated at sixth month and compared with baseline values. The detailed methods used for

TABLE I. Demographic and clinical features and mutations of patients with LRBA deficiency

Patient	Family	Current age (y)/sex	Consanguinity	AOO (mo)	Phenotype	Clinical diagnosis	Mutation	Outcome
P1	F1	13/M	+	18	RTI, CD, LP, ID	CVID	c.5047C>T, p.R1683*	Alive
P2	F1	9/F	+	48	CD, ID, LP	ALPS	c.5047C>T, p.R1683*	Alive
P3	F2	7/M	+	8	RTI, CD, LP	ALPS	c.7885delA, p.R2629fs	Alive
P4	F2	13/M	+	1	RTI, CD, LP, ID	ALPS	c.7885delA, p.R2629fs	Alive
P5	F3	26/M	+	3	RTI, CD, LP, ID	CVID	c.767+5_767+8delGTAT, p?	Alive
P6	F4	11/M	+	7	RTI, CD, LP, ID	CVID	c.2599C>T, p.Q867*	Alive
P7	F5	3/F	+	8	RTI, ID	IPEX-like	c.5172-2A>G, p?	Alive
P8	F6	14/M	+	6	RTI, CD, LP, ID	ALPS	c.1963C>T, p.R655*	Died
P9	F7	18/M	−	18	RTI, CD, ID	CVID	c.2836_2839delGAAA, p.E946*	Alive
P10	F8	35/F	+	84	RTI, CD, LP, ID	CVID	c.7238dupG, p.S2413Rfs*1	Alive
P11	F8	14/M	+	8	RTI, CD, LP	ALPS	c.7238dupG, p.S2413Rfs*1	Died
P12	F9	23/M	+	42	RTI, CD, ID	ALPS	c.2818dupC, p.Q940fs	Alive
P13	F10	18/M	−	72	RTI, CD, ID, LP	ALPS	c.1963C>T, p.R655*	Alive
P14	F11	16/F	+	4	RTI, CD, ID	CVID	c.2735_2738delGGGT, p.T912*	Alive
P15	F12	13/M	+	24	RTI, ID, LP	ALPS	c.3396-3397delAC, p.D975Yfs*15	Alive
P16	F13	11/M	+	13	RTI, ID, LP	IPEX-like	c.2496C>A, p.C832*	Alive
P17	F13	12/M	+	60	RTI, ID, LP	IPEX-like	c.2496C>A, p.C832*	Alive
P18	F14	16/F	+	18	RTI	CVID	c.5537C>T, p.S1846L	Alive
P19	F15	21/F	+	6	RTI, CD, ID, LP	CVID	c.7976C>G, p.S2659*	Alive
P20	F16	3.5//F	+	9	CD	IPEX-like	c.1496C>A, p.S499*	Alive
P21	F17	13/F	+	9	CD, ID	IPEX-like	c.3549_3550insA, p.A1184Sfs*34	Alive
P22	F17	4/M	+	—	—	Asymptomatic	c.3549_3550insA, p.A1184Sfs*34	Alive

ALPS, Autoimmune lymphoproliferative syndrome; *AOO,* age of onset; *CVID,* common variable immunodeficiency; *F,* female; *IPEX,* immune dysregulation, polyendocrinopathy, enteropathy, X-linked; *M,* male; *RTI,* respiratory tract infection.

flow cytometric and genetic analyses are described in this article's Online Repository at www.jaci-inpractice.org.

Abatecept therapy and clinical benefits

The detailed abatacept treatment courses were mentioned for each patient separately. The physicians were questioned for the effect of abatacept during follow-up. The degree of severity of each symptom was recorded as mild, moderate, or severe at baseline, 3rd, 6th, 9th, and 12th months and categorized as complete remission (CR), partial remission (PR), or nonresponsive according to the response to abatecept. Other immunosuppressants, which were used before and after abatacept, and abatacept side effects were recorded during the study.

Statistical analysis

Comparisons between groups were carried out using Student paired, unpaired, and 1-way ANOVA with Bonferroni posttest analysis, as indicated. Categorical variables were compared by χ^2 analysis. Receiver-operating characteristic test was used to determine the sensitivity and specificity. Differences in mean values were considered significant at a P value of less than .05.

RESULTS

Diagnosis of LRBA-deficient patients

Twenty-two genetically confirmed LRBA-deficient patients were included in this study. All patients had homozygous mutations, which were confirmed by Sanger sequencing (Table I). All analyzed patients (P1-7, P9, P10, and P12-19) had low LRBA protein expression (Figure 1, *A*). P8 and P11 died before flow cytometric evaluation, whereas samples from P20 to P22 could not be shipped for assessment. CTLA4 intracellular staining was evaluated in 6 patients and found to be lower than that in controls, as expected (Figure 1, *B*). To assess the performance of LRBA intracellular flow staining, we prospectively collected 89 samples referred to our center (genetically known LRBA-deficient patients [n = 17], patients presented as common variable immunodeficiency phenotype but had no LRBA mutation [n = 15], and healthy controls [n = 57]). The quantified mean fluorescence intensity (MFI) ratio, determined by dividing raw MFI by the background staining and then quantified by normalization to control MFI, was statistically lower in LRBA-deficient patients and higher in healthy controls (Figure 1, *C*). Then, to differentiate LRBA-deficient patients from others, receiver-operating characteristic analysis was performed and sensitivity and specificity were determined. The receiver-operating characteristic analysis yielded an area under curve of 0.98 with a 95% CI (0.96-1.00) (Figure 1, *D*). Our results revealed higher sensitivity (100%) and specificity (91.7%) by using a cutoff value of 0.76 for the quantified MFI ratio. This cutoff value was able to catch up all LRBA-deficient patients, and the positive predictive and negative predictive values were calculated as 73.9% and 100%, respectively.

Demographic characteristics and clinical presentations of LRBA-deficient patients

The patients' demographic characteristics and their salient clinical phenotypes are presented in Table I and are further detailed in this article's Online Repository at www.jaci-inpractice.org. There were 14 (63.6%) males and 8 (36.3%) females in our cohort. The mean age of the patients was 13.4 ± 7.9 years, and the follow-up period was 3.4 ± 2.3 years. The mean age at first symptoms was 24 ± 23 months, while the delay time in diagnosis was observed

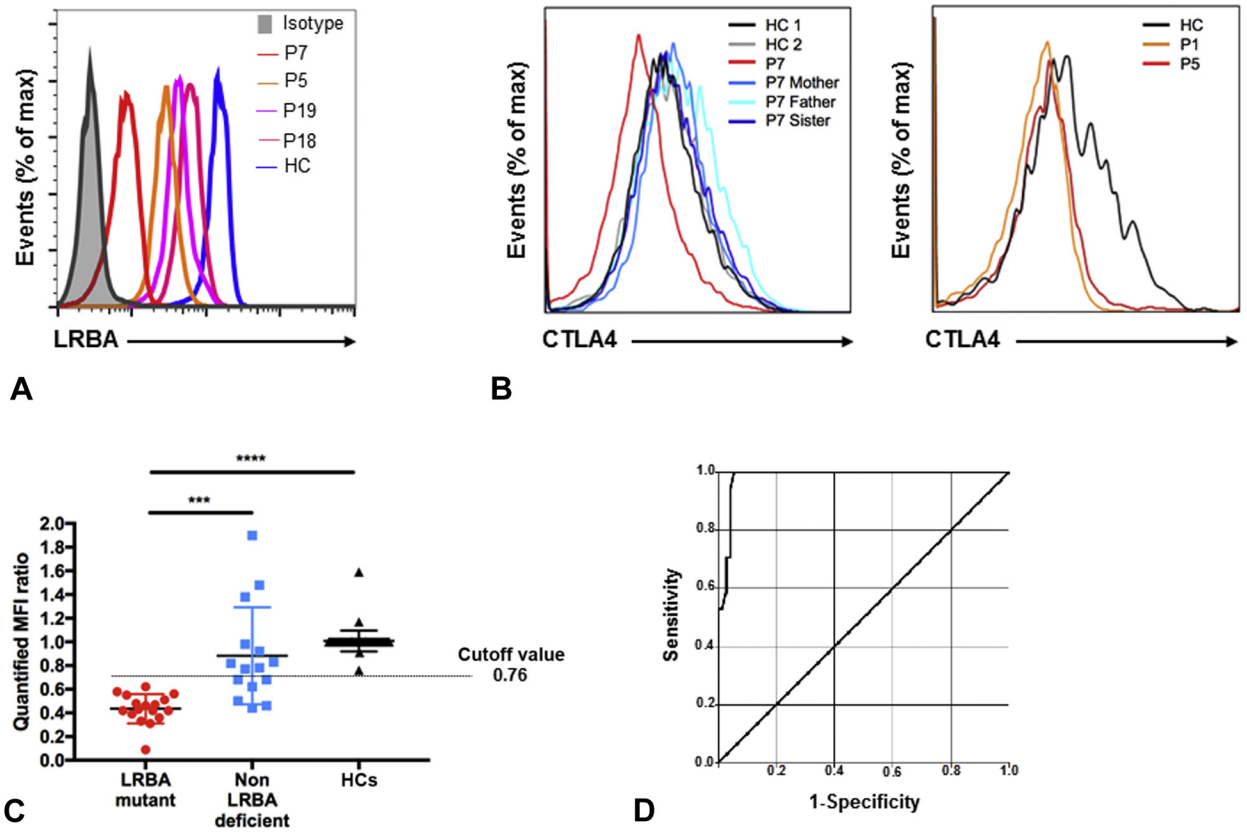

FIGURE 1. LRBA-deficient patients have low or absent LRBA and CTLA4 protein expression. (**A**) LRBA expression in lymphocytes from LRBA-deficient patients, P7 (*red line*), P5 (*orange line*), P19 (*purple line*), and P18 (*pink line*), compared with an HC (*blue line*). (**B**) Flow cytometric analysis demonstrates low CTLA4 expression on $CD4^{+}FOXP3^{+}$ regulatory T cells in LRBA-deficient patients P1, P5, and P7 compared with HCs and unaffected family members. (**C**) LRBA mutant patients (*red dots*) have significantly decreased MFI ratio of LRBA expression in PBMCs compared with non–LRBA-deficient (*blue dots*) and HCs (*black dots*). (**D**) Receiver-operating characteristic curve analysis shows the sensitivity and specificity of flow cytometric analysis for the detection of LRBA-deficient patients. The analysis was conducted on 17 mutation-verified LRBA-deficient patients, 15 patients who presented with an LRBA phenotype but had no LRBA mutation, and 57 HCs. Area under curve was yielded as 0.98 with a 95% CI (0.96-1.00). *FOXP3,* Fork-head box P3; *HC,* healthy control; *max,* maximum. $***P < .001$ and $****P < .0001$, Student unpaired 2-tailed *t* test.

as 9.5 ± 9.0 years. All patients had consanguinity except for P9 and P13. When the LRBA-deficient patients were evaluated according to their first clinical manifestations, 8 (36.3%) presented as common variable immunodeficiency, 8 (36.3%) as autoimmune lymphoproliferative syndrome, and 5 (22.7%) as immune dysregulation, polyendocrinopathy, enteropathy, X-linked–like disease (Table I). One asymptomatic patient (P22) was diagnosed in family screening. By the end of the study, 20 patients were alive, whereas 2 patients were deceased (P6 after HSCT and P11 due to severe disease course), with an overall survival of 91.0%.

Clinical phenotype of LRBA-deficient patients

The clinical phenotype of LRBA-deficient patients mainly consisted of recurrent infections (n = 19 [86.4%]), immune dysregulation (ID) (n = 18 [81.8%]), and LP (n = 16 [72.7%]). The other common manifestations related to LRBA deficiency were failure to thrive (n = 13 [59.1%]), osteoporosis (n = 6 [27.3%]), finger clubbing (n = 5 [22.7%]), cholelithasis (n = 5 [22.7%]), deafness (n = 3 [13.6%]), malignancy (n = 2 [9.1%]), chronic glomerulonephritis (n = 2 [9.1%]), pulmonary thromboembolism (n = 1 [4.5%]), and cholecystitis (n = 1 [4.5%]). The clinical presentations of the patients are summarized in Figure 2, Table E1 (in this article's Online Repository at www.jaci-inpractice.org), and Online Repository.

ID was the second predominant feature in our cohort. Most patients had chronic diarrhea (CD) (n =18 [81.8%]) and enteropathy was proven by biopsy in 14 (63.6%) patients. Villus atrophy, intraepithelial lymphocytosis, lymphoid hyperplasia and aggregates, chronic gastritis and duodenitis, active colitis, and eosinophilic infiltration were frequently observed. Autoimmune hemolytic anemia and immune thrombocytopenia were the most common hematological manifestations, observed in 9 (40.9%) and 11 (50%) patients, respectively. Because of autoimmune hemolytic anemia, choledocholithiasis was observed in 5 (22.7%) patients. Six patients (P8, P12, P13, P15, P17, and P19) developed Evans syndrome, which was usually intractable and resistant to treatment with immunosuppressive drugs. It was controlled after HSCT in P8, splenectomy in P12, and abatacept in P17 and P19. Three patients (13.6%; P1, P7, and P21) had type 1 diabetes mellitus (DM), requiring regular insulin

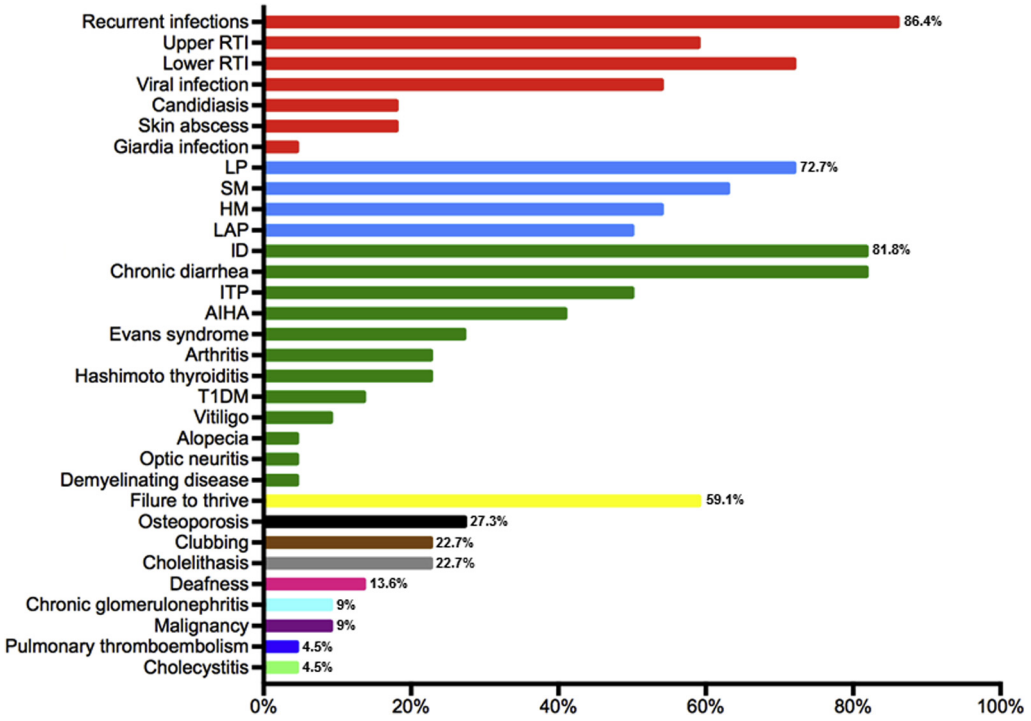

FIGURE 2. Clinical features of LRBA-deficient patients. The bars are depicted as percentages. The disease symptom clusters are indicated with different colors. Red bars show infections, blue bars denote LP, green bars demonstrate ID, yellow bar shows failure to thrive, black bar indicates osteoporosis, brown bar represents clubbing, silver bar indicates cholelithasis, pink bar shows deafness, and light blue, purple, dark blue, and light green bars indicate chronic glomerulonephritis, malignancy, pulmonary thromboembolism, and cholecystitis, respectively. *AIHA,* Autoimmune hemolytic anemia; *HM,* hepatomegaly; *ITP,* immune thrombocytopenia; *LAP,* lymphadenopathy; *RTI,* respiratory tract infection; *SM,* splenomegaly; *T1DM,* type 1 diabetes mellitus.

injections. During the course of the study, 5 (22.7%) patients (P5, P6, P9, P12, and P21) had arthritis without a discernable etiology. Other less frequent autoimmune features were alopecia (P3), vitiligo (P6 and P9), Hashimoto thyroiditis (P3, P4, P5, P10, and P16), and optic neuritis and demyelinating disease (P12).

The third common manifestation in LRBA-deficient patients was LP, which was characterized by splenomegaly (n = 14 [63.6%]), hepatomegaly (n = 12 [54.5%]), and lymphadenopathy (n = 11 [50%]).

Immunologic phenotype of LRBA-deficient patients

Immunologic data were available on all 22 patients. During the time of evaluation, lymphopenia (n = 6 [27.2%]), neutropenia (n = 1 [4.5%]), anemia (n = 3 [13.6%]), and thrombocytopenia (n = 8 [36.3%]) were recorded. Serum immunoglobulin levels before intravenous immunoglobulin therapy were available in 22 patients and showed low IgG in 10 (45.5%), low IgM in 12 (54.5%), and low IgA in 16 (72.7%) patients. The rest of the patients had normal or high immunoglobulin levels (see Table E2 in this article's Online Repository at www.jaci-inpractice.org). Extensive flow cytometric analysis including T, B, natural killer (NK), and T-cell and B-cell subtypes were investigated and summarized in Figure E1 and Table E2 in this article's Online Repository at www.jaci-inpractice.org. Reduced $CD3^+$ T-cell counts were observed in 6 (27.2%) patients, whereas 3 (13.6%) patients had an increased value. The $CD4^+$ and $CD8^+$ T cells were low in 7 (31.8%) and 8 (32%) patients, respectively. Double-negative T-cell percentages were increased in 7 (33.3%) patients; all of them were considered as autoimmune lymphoproliferative syndrome initially. B-cell compartment showed abnormalities characterized by reduced total B cells in 45.5%, increased naive ($CD27^-IgD^+$) cells in 27.7%, reduced class-switched memory ($CD27^+IgD^-$) cells in 63.1%, and increased activated B cells ($CD21^{low}CD38^{low}$) in 30.7% of patients. NK cells were found to be low in 8 (36.3%) patients.

Mutational analysis and genotype-phenotype correlations

A schema depicting the LRBA mutation sites is shown in Figure E2, *A*, in this article's Online Repository at www.jaci-inpractice.org. All the patients had homozygous mutations and consanguinity was prominent (except P9 and P13) in our cohort. Mutations of P1, P2, P3, P4, P7, P8, P13, P16, and P17 were described previously.[1,7,10,15-17] P5, P6, P7, P9, P10, P11, P12, P14, P15, P18, P19, P20, P21, and P22 had novel mutations. Most of the mutations were nonsense and frameshift, but 2 mutations (P5 and P7) were splice junction type (Table I). cDNA analysis confirmed that both splice-site mutations resulted in exon aberrant splicing, which was associated with low LRBA protein expression as detected by immunoblotting and flow cytometry analyses (Figure 1, *A*; see also Figure E2, *B* and *C*). Further analysis pointed to identical LRBA mutations giving rise to divergent clinical outcomes, even among siblings. Overall,

TABLE II. Abatacept treatment courses and clinical responses of LRBA-deficient patients

Patients	Time of usage (mo)	Clinical indication for abatacept	Initial dose	Maintenance dose during follow-up or transplantation	Remission rate and time (mo)	Final status
P1	15	LP	15 mg/kg/1 wk	Switched to per 2 wk (at 6th mo of therapy)	LP (CR): 6th mo	Insulin-dependent DM continued
		Diarrhea			Diarrhea (CR): 3rd mo	
		DM			DM (NR)	
P2	7	LP	15 mg/kg/1 wk	Switched to per 2 wk (at 2nd mo of therapy)	LP (CR): 3rd mo	Symptoms are controlled
		Diarrhea			Diarrhea (CR): 3rd mo	
		ITP			ITP (CR): 3rd mo	
P3	33	LP	10 mg/kg/4 wk	Switched to 20 mg/kg/2 wk (at 4th mo of therapy)	LP (CR): 4th mo	CR of diarrhea was achieved at 3rd mo of maintenance therapy
		Diarrhea			Diarrhea (PR): 10th mo	
P4	33	LP	10 mg/kg/4 wk	10 mg/kg/4 wk	LP (CR): 6th mo	Alopecia is partially controlled
		Diarrhea			Diarrhea (CR): 6th mo	
		Alopecia			Alopecia (PR): 12th mo	
P5	9	LP	20 mg/kg/2 wk	HSCT (at 6th mo of therapy)	LP (CR): 3rd mo	Alive with 98% chimerism
		Diarrhea			Diarrhea (CR): 3rd mo	
		ITP			ITP (PR): 6th mo	
P7	25	Respiratory tract infections	20 mg/kg/4 wk	20 mg/kg/4 wk	DM (NR)	Insulin-dependent DM continued
		DM				
P9	5	LP	10 mg/kg/1 wk	HSCT (at 5th mo of therapy)	LP (CR): 2nd mo	Alive with 98% chimerism
		Diarrhea			Diarrhea (CR): 2nd mo	
		AIHA			AIHA (CR): 2nd mo	
P10	12	LP	10 mg/kg/4 wk	10 mg/kg/2 wk	LP (CR): 6th mo	CR of diarrhea was achieved at 1st mo of maintenance therapy
		Diarrhea			Diarrhea (PR): 6th mo	
P12	12	LP	15 mg/kg/2 wk	15 mg/kg/2 wk	LP (CR): 2nd mo	Symptoms are controlled except for demyelinating disease
		Diarrhea			Diarrhea (CR): 2nd mo	
		Arthritis			Arthritis (CR): 2nd mo	
		Demyelinating disease			Demyelinating disease (NR)	
P14	7	Diarrhea	10 mg/kg/2 wk	Switched to per 4 wk (after 3 doses)	Diarrhea (CR): 4th mo	Symptoms are controlled
P16	11	Diarrhea	10 mg/kg/2 wk	10 mg/kg/2 wk	Diarrhea (CR): 5th mo	Symptoms are controlled
P17	18	Evans syndrome	10 mg/kg/2 wk	10 mg/kg/2 wk	Evans syndrome (CR): 3rd mo	Symptoms are partially controlled for LP, diarrhea, and GILID
		LP			LP (PR): 6th mo	
		Diarrhea			Diarrhea (PR): 4th mo	
		GILID			GILID (PR): 6th mo	
P18	5	Respiratory tract infections	10 mg/kg/2 wk	Switched to per 4 wk (after 3 doses)	—	No autoimmunity at the beginning and during follow-up

P19	18	Evans syndrome Diarrhea LP	10 mg/kg/2 wk	Switched to per 4 wk (after 3 doses)	Evans syndrome (CR): 6th mo Diarrhea (PR): 4th mo LP (NR)	Diarrhea is partially controlled, LP is not responding to therapy
P20	21	Diarrhea	10 mg/kg/2 wk	Switched to per 4 wk (after 3 doses)	Diarrhea (CR): 6th mo	Diarrhea is controlled
P21	12	Diarrhea	10 mg/kg/2 wk	Switched to per 4 wk (after 3 doses) HSCT (at 12th mo of therapy)	Diarrhea (CR): 3rd mo Arthritis (PR): 6th mo DM (NR)	Alive with 98% chimerism

AIHA, Autoimmune hemolytic anemia; *GILID*, granulomatous-lymphocytic interstitial lung disease; *ITP*, immune thrombocytopenia; *NR*, nonresponsive.

there were no strong genotype-phenotype relationships that governed the manifestations of LRBA deficiency in our patient population.

Medications and disease course

Because of the recurrent infections, hypoglobulinemia, and ID, 17 (77.2%) patients received prophylactic antibiotics and all patients were on immunoglobulin replacement (2 of them with subcutaneous preparations). Two of 17 (11.2%) patients were placed on azithromycin, whereas the rest (88.2%) were on trimethoprim-sulfamethoxazole prophylaxis. Because of candidiasis, fluconazole prophylaxis was given to P1, P5, P16, P17, and P21. Acyclovir prophylaxis was used in P16, P17, and P18 (Table E1). HSCT was performed in P6, P8, P9, and P21.

To control immune dysregulatory features observed in the patients, various mono or combination therapies with immunosuppressive agents were started as follows: abatacept (n = 18 [81.8%]), prednisolone (n = 12 [54.5%]), mycophenolate mofetil (n = 5 [22.7%]), cyclosporine A (n = 4 [18.1%]), sirolimus (n = 2 [9%]), sulfasalazine (n = 1 [4.5%]), azathioprine (n = 1 [4.5%]), adalimumab (n = 1 [4.5%]), and hydroxychloroquine (n = 1 [4.5%]). Splenectomy was performed in 3 patients because of uncontrolled LP (P11 and P15) and Evans syndrome (P12). Five patients (P5, P6, P8, P9, and P21) had a transplant because of the advanced disease and poor response to treatment. More specifically, the indications for the transplantation were persistent hematological findings (P5, P6, and P8), uncontrolled LP (P6), CD (P6 and P21), and severe side effects of immunosuppressants (P9). Various donors were used, including matched related donors (P5 and P8), matched unrelated donors (P6 and P9), and mismatched unrelated donor (P21). Myeloablative regimens were applied in P5, P8, P9, and P21, whereas reduced-intensity conditioning regimen was applied in P6. Decisions about the choices of applied conditioning regimens were made by the respective institutions. P6 was deceased after transplant because of acute graft versus host disease and sepsis, whereas P5, P8, P9, and P21 are still alive, with chimerism between 95% and 98% of donor cells.

Patients were followed up prospectively during abatacept treatment (P1-P7, P9, P10, P12, and P14-P21). The median duration of abatacept therapy was 12.5 months (range, 5-33 months). The main clinical phenotypes of the 18 patients at the start of abatacept therapy were categorized as ID, CD, and LP. Remission after abatacept therapy was calculated for each phenotype (P6 and P15 were not evaluated because of the short-term duration of abatacept therapy in their case). All patients responded to abatacept therapy but to different degrees (Table II). ID symptoms were present in 13 patients, and 5 (38.4%) patients showed CR, 4 (30.7%) patients had PR, and 4 (30.7%) were nonresponsive. CD was observed in 14 patients and CR was observed in 11 (78.5%), whereas 3 (21.5%) of these patients had PR. LP was determined radiologically in 10 patients and followed regularly during abatacept treatment. At the end of the study period, CR in 8 (80%), PR in 1 (10%), and no response in 1 (10%) patient were achieved. As a result, although some patients received abatacept for a short period during the study (P6 and P9 due to HSCT and P14 due to short follow-up), the best CR was achieved for LP and followed by CD and ID (Figure 3, *A*). In all patients, at least 1 of the symptoms was completely or partially controlled and there was no unresponsiveness to abatacept. Among the ID manifestations,

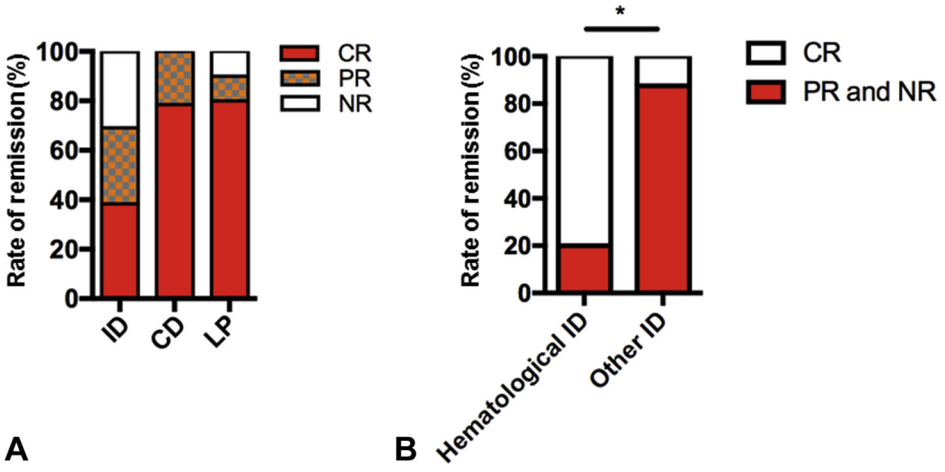

FIGURE 3. LRBA disease symptoms display different responses to abatacept treatment. (**A**) The remission rates for ID, CD, and LP. (**B**) The comparison of the remission rates of hematological ID (autoimmune hemolytic anemia, immune thrombocytopenia) vs other immune dysregulatory symptomatologies (diabetes, alopecia, arthritis, demyelinating disease, granulomatous-lymphocytic interstitial lung disease). The remissions are indicated as complete (CR), partial (PR), or nonresponsive (NR). The bars are presented as percentages. $*P < .01$, χ^2 test.

autoimmune hemolytic anemia and immune thrombocytopenia were statistically the most frequently controlled manifestations (Figure 3, *B*). Type 1 DM was not reversible after abatacept (P1, P7, and P21).

Different centers used various abatacept therapy regimens for the patients (Table II), and these were compared with each other to determine which therapy frequency was the most efficient in controlling disease symptoms. Patients on abatacept every week or every other week attained CR in a shorter time compared with patients on abatacept every 4 weeks (Figure 4, *A*). When every week and every other week regimens were compared, all symptoms were completely controlled in 1-week therapy option, whereas 2-week regimens resulted in partial control of some symptoms (Figure 4, *B*, and Table II).

At the end of the study, only 2 patients were still on other immunosuppressive drugs (steroids and mycophenolate mofetil in P16 and steroids in P19) with tapered dosing. Notably, 6 patients (P1, P5, P9, P12, P20, and P21) were able to stop their steroids. P1 and P12 stopped their sirolimus therapy, whereas P12 also stopped adalimumab and sulfasalazine therapy. P2 and P19 were on mycophenolate mofetil and cyclosporine, but discontinued both drugs during treatment with abatacept. P5 was able to stop local budesonide, which was used for colitis. Intravenous immunoglobulin therapy was not discontinued in any patient during the study. Two patients (P2 and P3) suffered from side effects such as mild eczema and dermatophyte infection, which were tolerated well and managed without cessation of abatacept therapy. P16 had severe oral and esophageal candidiasis and fungal pneumonia after the first dose of abatacept, leading to cessation of treatment. However, it was reinitiated after 9 months without side effects.

Abatacept and immunologic changes

The cellular immunologic changes associated with abatacept therapy were serially evaluated by flow cytometry. Abatacept had the most prominent impact on patients' naive T cells ($CD4^+CD45RA^+$ and $CD8^+CD45RA^+$), which significantly increased on therapy, whereas no difference was observed in memory T cells (see Figure E3, *A-D*, in this article's Online Repository at www.jaci-inpractice.org). There were no changes after abatacept therapy in $CD3^+$ T cells, B-cell subtypes, and NK cells (see Figure E4, *A-D*, in this article's Online Repository at www.jaci-inpractice.org). The baseline $CD4^+PD1^+CXCR5^+$ cT_{FH} cells were evaluated in 10 (45.4%) patients and were significantly higher compared with those in healthy matched controls (Figure 5, *A* and *B*). As previously described,[15] LRBA-deficient patients had more activated cT_{FH} cells as demonstrated by increased expression of programmed cell death-1 (Figure 5, *C* and *D*). Baseline programmed cell death-1^+ cT_{FH} cell profile was compared with cT_{FH} counts at sixth month posttherapy initiation (P1, P5, P7, P9, P10, P12, P14, P18, and P19). There was a decrease in the frequencies and activation profile of $CD4^+PD1^+CXCR5^+$ cT_{FH} cells on abatacept therapy, with normalization of cT_{FH} cells observed in most LRBA-deficient patients (P1, P7, P9, P10, P12, P14, and P18) (Figure 5, *E* and *F*).

Two patients (P5 and P19) persisted in having elevated cT_{FH} cells despite abatacept therapy given every other week (Figure 6, *A* and *B*). Therefore, we investigated the distinguishing clinical and immunologic features of those 2 patients. Both had statistically longer disease course (4.2 ± 2.1 years) compared with others (2.1 ± 1.1 years). Interestingly, P5 and P19 also had a more severe disease course as compared with other patients, although they responded to some extent to the abatacept treatment (Table II; see also Table E3 in this article's Online Repository at www.jaci-inpractice.org). P5 had gastric adenocarcinoma, failure to thrive, LP, diarrhea, and pancytopenia. After 9 months of therapy, although the LP and CD were controlled, thrombocytopenia was persistent and his cT_{FH} number increased during the therapy (Figure 6, *A* and *B*). Therefore, he had a transplant from his fully matched healthy sibling as a donor, and is now doing well at sixth month of transplantation, with 96% donor chimerism. His cT_{FH} number and LRBA expression were normalized after HSCT (Figure 6, *B* and *C*). P19 had early onset Evans syndrome, LP, and

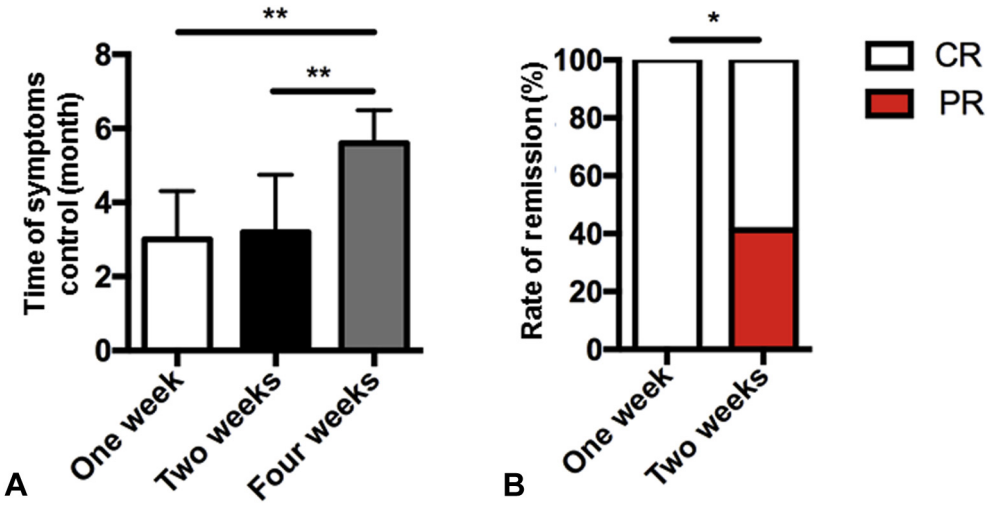

FIGURE 4. Various CR rates in LRBA-deficient patients after treatment with abatacept according to dosing interval. (**A**) The CR rates in patients who received abatacept with 1- or 2-week interval in comparison to 4 weeks. (**B**) The percentages of CR or PR rate in LRBA-deficient patients in terms of dose interval. $*P < .05$, χ^2 test. $**P < .01$, 1-way ANOVA test.

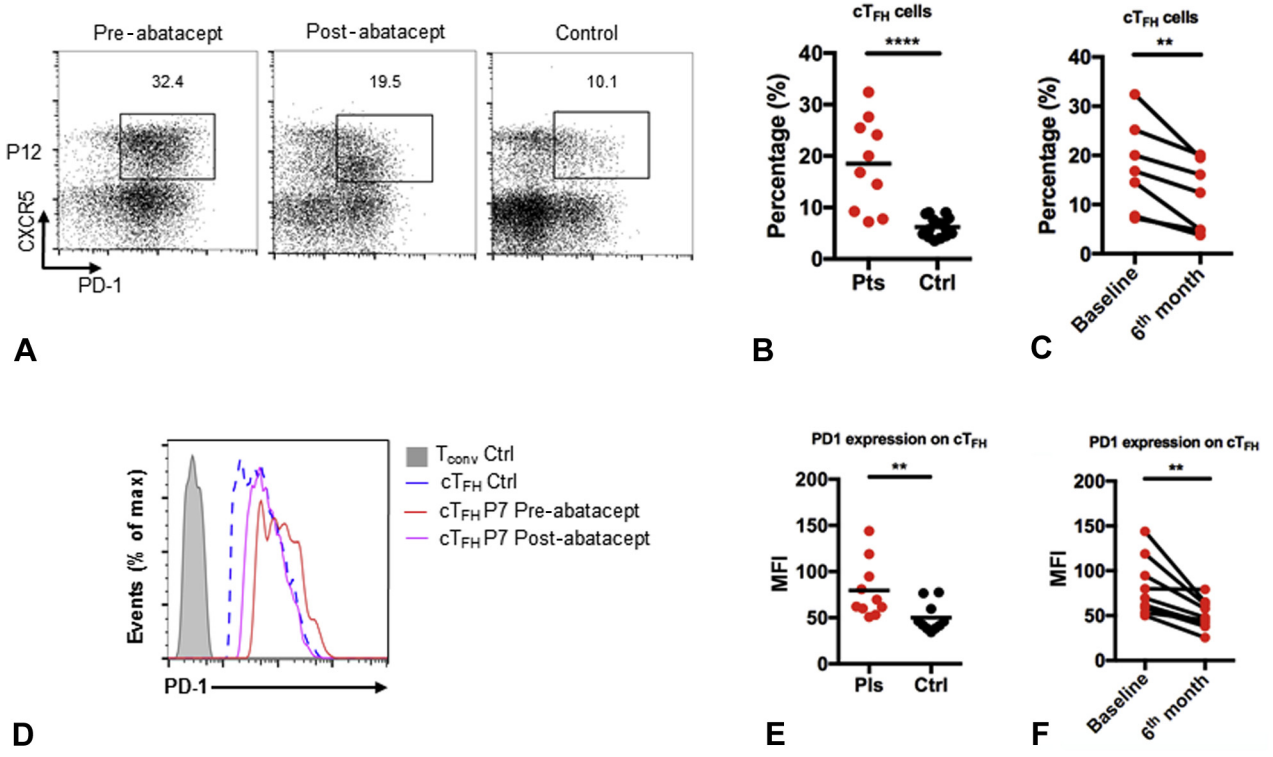

FIGURE 5. LRBA-deficient patients have increased activated cT_{FH} cells at baseline, which are normalized after abatacept treatment. Flow cytometric analysis of CXCR5 and PD-1 expression in $CD4^+$ Tcells in LRBA-deficient patients before (**A, B**) and after (**A, C**) treatment with abatacept. PD-1 expression on patients' cT_{FH} cells before (**D, E**) and after (**D, F**) abatacept treatment. *Ctrl,* Controls; *max,* maximum; *Pts,* patients; *PD-1,* programmed cell death-1. $****P < .0001$, $**P < .01$, Student unpaired and paired 2-tailed *t* test.

CD. Although her hemolysis was controlled and diarrhea decreased over time, LP and inflammatory bowel disease did not respond well to abatacept. Currently, she is at 20th month of therapy. Immunologically, these 2 patients with high cT_{FH} numbers exhibited significantly low baseline lymphocyte, $CD3^+$, $CD19^+$, and NK-cell counts compared with patients who had decreased cT_{FH} cells after abatacept (P1, P7, P9, P10, P12, P14, and P18). They also demonstrated more

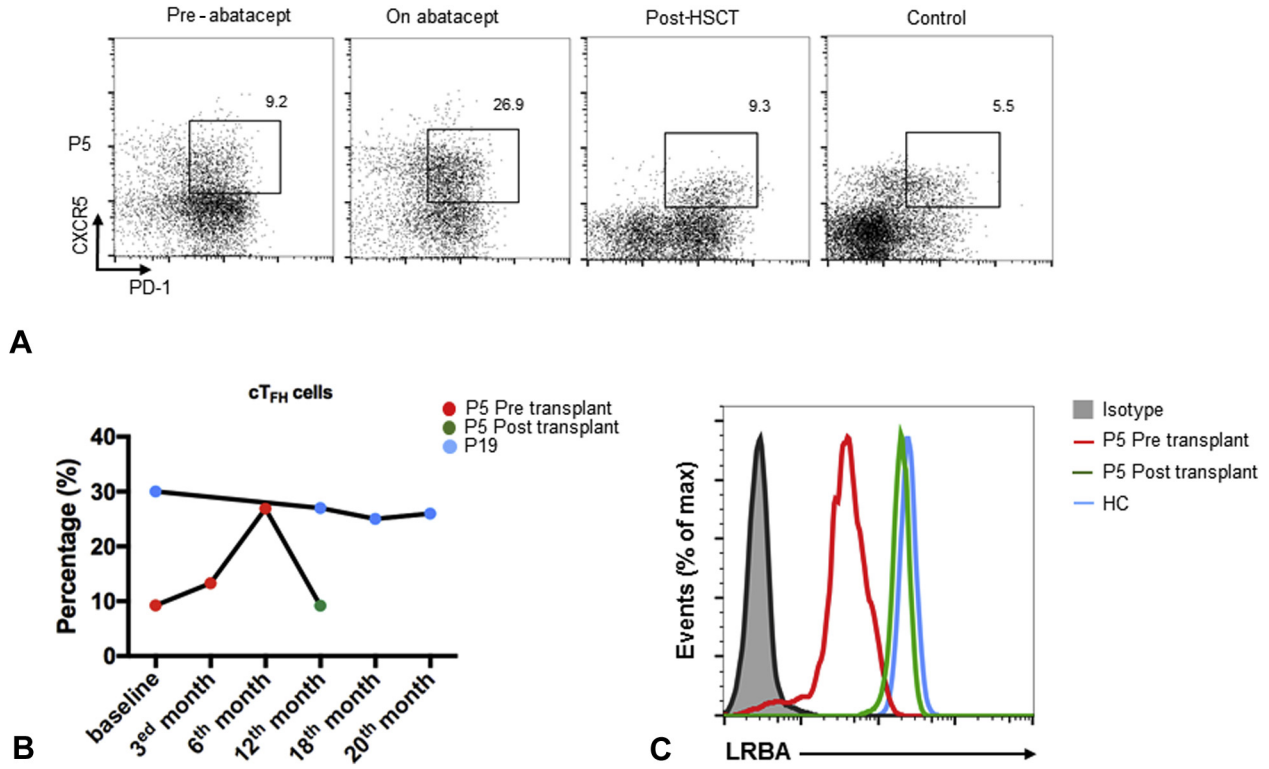

FIGURE 6. The cT$_{FH}$ cells guide the disease activity in LRBA-deficient patients. (**A**) Flow cytometric analysis of CXCR5 and PD-1 expression in CD4$^+$ T cells in patient (P5) at baseline, on abatacept, and after HSCT compared with the HC. (**B**) The change in cT$_{FH}$ cell percentages in LRBA-deficient patients on abatacept (P5 and P19) and after transplantation (P5). (**C**) The LRBA protein expression in P5 after transplant compared with the baseline level and HC. *HC,* Healthy control; *max,* maximum; *PD-1,* programmed cell death-1.

dysregulated phenotype characterized by inverted naive to memory CD4$^+$ cells (Table E3).

DISCUSSION

In this report, we prospectively evaluated 22 LRBA-deficient patients from different immunology centers in Turkey. Our results provided a comprehensive long-term evaluation of clinical and immunologic characteristics of LRBA-deficient patients. Patients presented with various phenotypes such as early onset respiratory tract infections, ID, and LP. Our results showed for the first time the long-term benefits of targeted CTLA4-immunoglobulin therapy in LRBA deficiency. Abatacept showed the best CR for LP followed by CD and ID symptoms. Interestingly, more favorable responses were achieved for hematological ID symptoms compared with other ID symptoms, whereas type 1 DM was not controlled well with abatacept. Our results also demonstrated the efficacy of dosing intervals used for patients. Receiving abatacept at 1- or 2-week interval exhibited more disease control compared with 4-week regimen. Using the CD4$^+$PD1$^+$CXCR5$^+$ cT$_{FH}$ cells as a biomarker for the disease control over time, we showed for the first time that a few patients did not respond well to abatacept and were found to have persistently high cT$_{FH}$ cells during the study. Those patients were noted to clinically have more disease burden, which was characterized immunologically by lymphopenia, with low total T, B, and NK cells.

Flow cytometric analysis revealed that in our cohort, mutations that decreased or abolished LRBA protein expression were present in 17 of 22 patients. Notably, near-normal expression of LRBA protein could be detected by flow cytometry even in patients with early stop gained mutation, which can lead to the underestimation of the diagnosis.[7,18] Therefore, as described previously, using an MFI threshold would be supportive for determination of all LRBA-deficient patients.[18] The calculated MFI ratio cutoff point to determine our normative reference data for LRBA expression was 0.76. This ratio was able to detect all LRBA-deficient patients with high sensitivity (100%) and specificity (91.7%). In this assay, we determined the LRBA protein expression in PBMCs without stimulation, in contrast to the Gamez-Diaz et al[18] study, which detected LRBA protein expression in stimulated PBMCs. Similar to the aforementioned study, we also found 6 patients with low protein expression but without identifiable mutations in *LRBA*. Therefore, we suggest that when evaluating LRBA protein expression by flow cytometry, each laboratory involved in such assays derive its own MFI ratio cutoff point to aid in the identification of LRBA deficiency, rather than use raw MFI values for such purposes.

Recently, abatacept, a T-cell modulator, has been proposed as a targeted precision therapy for LRBA-deficient patients. Abatacept mimics the function of the cellular CTLA4 pool, rendered missing by LRBA deficiency, in negatively regulating the immune responses by blockading or capturing CD80/86 molecules found on antigen-presenting cells.[1,13,15] Patients have been reported to generally respond well to abatacept therapy, with

decreased disease symptomatology related to the lung infiltrations, LP, CD, and autoimmune features.[1,15] In this study, the relatively large patient number prospectively treated with abatacept allowed for better resolution of the responses of the different phenotypes of the disease to therapy. Thus, the best clinical benefit was achieved for LP, followed by CD and ID. Of note, in all patients at least 1 disease feature responded to the therapy. Interestingly, the hematological autoimmune features responded better compared with other ID symptoms. Abatacept therapy did not reverse type 1 DM, possibly because of terminal damage of the pancreatic beta islets inflicted by the autoimmunity.

One important question about abatacept therapy in LRBA deficiency that we addressed in our cohort involved its optimal dosing frequency. In previous reports, different dosing intervals, ranging from every 2 to 4 weeks, were used.[1,13] Importantly, the response of individual disease attributes, including ID, CD, and LP, to abatacept therapy was not differentially analyzed. We therefore compared different treatment regimens offered to the patients, and found that abatacept given at every 1- or 2-week interval provided better disease control and faster achievement of CR as compared with every 4-week regimen. Interestingly, the 1-week regimen came out with CR for all symptoms described. Our results also support the utility of abatacept as a bridge therapy in preparation of HSCT, with the potential for improved transplant outcomes as observed for P5 and P9.

In our study, we used cT_{FH} cell frequencies as a biomarker for monitoring disease activity. As demonstrated previously by our group, the strikingly increased $CD4^+PD1^+CXCR5^+$ cT_{FH} cells found in LRBA-deficient patients sharply declined after abatacept therapy in most patients.[15] Nevertheless, they continued to be persistently high in 2 patients, both of whom had a more severe disease course compared with those patients whose cT_{FH} cell frequencies normalized. Although some of their disease parameters responded to the abatacept treatment, those 2 patients had a more protracted overall disease activity and demonstrated significant lymphopenia accompanied by low $CD3^+$, $CD19^+$, and NK cells. They also had a more dysregulated immune phenotype characterized by inverted naive to memory $CD4^+$ cells. Thus, persistently high cT_{FH} cells may suggest relative resistance to abatacept therapy, requiring a modified therapeutic approach.

CONCLUSIONS

The presented large cohort provided a prospective evaluation of LRBA-deficient patients during abatacept treatment. The targeted therapy was able to effectively control the different immune dysregulatory disease manifestations in most patients, and more favorable responses were achieved in patients who received abatacept at weekly intervals without serious side effects. Monitoring cT_{FH} cells during abatacept therapy provides a useful measure of disease activity, and may uncover cases of relative therapy resistance that require alternative treatment approaches.

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ONLINE REPOSITORY

METHODS

Antibodies and flow cytometry

To determine lymphocyte subsets, the following mAbs were used: fluorescein isothiocyanate (FITC)-conjugated anti-CD3, allophycocyanin (APC)-Alexa Fluor 700 (APC-A700) CD4, Krome Orange (KO) CD45, Alexa Fluor 750 (APC-A750) CD45RA, phycoerythrin (PE) CD197 (CCR7), Pacific Blue (PB) IgM, APC TCRαβ, PE-conjugated anti-CD4, pycoerythrin-cyanin (PC) 7 CD8, APC-A700 CD14, PE CD16, PC5.5 CD56, APC-A750 CD19, PB CD20, CD21, PB CD31, PC5.5 CD38, phycoerythrin-Texas Red-x (ECD) CD45RO, FITC IgD, PC5.5 TCRγδ, and PC7 HLA-DR. All antibodies were purchased from Beckman Coulter (Indianapolis, Ind). For lymphocyte subset analysis, 100 μL of whole blood was incubated with mAbs against surface markers for 20 minutes in the dark at room temperature. Red cells were then lysed and washed before acquisition. In intracellular staining for LRBA, the PBMCs were isolated by Ficoll-Hypaque density gradient centrifugation, fixed, permeabilized (BD Biosciences, Pharmingen, San Diego, Calif), and incubated with 1:100 antihuman rabbit LRBA polyclonal antibody (Sigma-Aldrich, Gallen, Switzerland) for 30 minutes at 4°C and further stained with a 1:500 dilution of secondary FITC-labeled antirabbit IgG antibody (Thermo Fisher Scientific, San Diego, Calif) for 30 minutes at 4°C. The ratio of MFI for intracellular LRBA was determined by dividing raw MFI by the background staining, measured by using only secondary antibody. Patient samples were analyzed concurrently with control samples. Finally, patients' MFI ratio was quantified by dividing with the control MFI ratio.

Total intracellular CTLA4 levels were determined from PBMCs by fixing and permeabilizing (eBioscience FoxP3 staining kits, San Diego, Calif) the cells and then staining for CTLA4 (BD Biosciences). Staining for cT_{FH} cells was performed on freshly isolated PBMCs, and surface staining for CD4 (BD Biosciences), programmed cell death-1, and CXCR5 (Biolegend, San Diego, Calif) was performed for 15 minutes in the dark at room temperature. Data were collected with a Navios EX cytometer (Beckman Coulter) and analyzed with FlowJo software (TreeStar, Ashland, Ore).

Sanger sequencing analysis

LRBA sequence was amplified from genomic DNA by PCR and sequenced bidirectionally using dye-terminator chemistry. Primers used in amplification reactions are available on request.

Immunoblotting, RNA isolation, and cDNA synthesis

Protein lysates derived from lymphocytes were separated by means of SDS-PAGE, transferred to nitrocellulose membranes, and immunoblotted with a polyclonal rabbit anti-LRBA antibody (Sigma-Aldrich, St Louis, Mo). The blots were reprobed with polyclonal rabbit anti-dedicator of cytokinesis 8 antibody (Sigma-Aldrich). Band intensities were quantified with ImageJ software (National Institutes of Health, Bethesda, Md). RNA was extracted from activated T cell using RNeasy Mini Kit (Qiagen, Germantown, Md), followed by in-solution DNAse digestion using Qiagen's RNAse-Free DNAse Set and subsequent cleanup using RNeasy Mini Elute kit. cDNA was then synthesized using SuperScript II Reverse Transcriptase kit (Invitrogen, Carlsbad, Calif), combined with OligoDT primers. Synthesized cDNA was amplified via PCR using Thermo's PCR Master Mix (2×). Primers were rooted in the indicated exons. PCR product was run through a 2% agarose gel.

Detailed history of patients and findings

Patient P1, a 13-year-old boy, who was born to consanguineous parents, was referred to the clinic because of recurrent lower respiratory tract infection (LRTI) and CD since age 1.5 years. During follow-up, he developed LP, type 1 DM, failure to thrive, and autoimmune thrombocytopenia. He had swollen lymph nodes with reactive lymphoid hyperplasia and EBV positivity. The physical examination showed severe growth failure, hepatosplenomegaly, enlarged axillar, cervical, and inguinal lymph nodes, clubbing, and perforated tympanic membranes. He was receiving intravenous immunoglobulin (IVIG) because of low IgG, IgM, and IgA levels. Lymphocyte-subset analysis demonstrated inverted CD4/CD8 ratio, low naive $CD4^+$ T cells, and low class-switched memory B cells. Total villous atrophy and intraepithelial lymphocytosis with predominance of $CD3^+$ T cells were striking on intestinal biopsy. Sirolimus and methyl prednisolone were initiated to control the LP and diarrhea. Although stool frequency decreased after therapy, the response could not be sustained. Genetic analysis revealed homozygous mutation in *LRBA* (c.5047C>T, p.R1683*). Abatacept was initiated with a dose of 15 mg/kg every week. At the third month, he showed great improvement, with a total recovery from diarrhea and he gained weight. At the sixth month, sirolimus was stopped because of complete regression of LP (only mild hepatomegaly detected) and the dose interval of abatacept was switched to every 2 weeks. At the 15th month of abatacept therapy, his weight was noted to be appropriate according to his age.

Patient P2 (now 9 years old) was born to consanguineous parents and presented with thrombocytopenia at age 5 years. She is a cousin of P1. She was treated with pulse steroid and IVIG. She also had concomitant LP. At age 6 years, when she was on low-dose steroid, she started to have diarrhea, with a frequency of 4 to 5 times per day. Her colonoscopic examination showed colitis and Crohn disease was diagnosed. Autoimmune lymphoproliferative disease was thought because of chronic LP and immune thrombocytopenia (ITP). At age 8 years, she was hospitalized because of vomiting and headache and cranial magnetic resonance imaging showed left subdural hematoma. The intracranial bleeding was secondary to the severe ITP. Later, she was started on high-dose IVIG and cyclosporine. However, because of reluctant thrombocytopenia, romiplastim and MMF were added. Although she used multiple immunosuppressants, her ITP was persistent. Therefore, genetic analysis was conducted and the same mutation of P1 was detected. Afterwards, abatacept was initiated at 15 mg/kg/wk. After 2 months of therapy, her thrombocyte count had increased gradually and after 4 months it was found to be above $100{,}000/mm^3$. Hereafter, the dose was decreased to 15 mg/kg every 2 weeks. Meanwhile, at the second month of therapy, diarrhea and LP were completely controlled and other immunosuppressants were tapered. Currently, she is at seventh month of therapy with a good clinical status.

Patient P3 is a 7-year-old Turkish boy, a product of consanguineous marriage, who had multiple hospital admissions since the age of 9 months for recurrent respiratory tract infection (RTI) and intermittent nonbloody diarrhea. He also had

hepatosplenomegaly and pancytopenia without a clear diagnosis. His upper gastrointestinal endoscopic imaging showed villous atrophy and intraepithelial lymphocyte infiltration. Despite a trial of a gluten-free diet, no clinical or histopathologic improvement was observed. Immunologic evaluation showed mild low IgM and normal lymphocyte subsets. Frameshift deletion homozygous mutation (c.7885delA, p.R2629fs) in LRBA was detected, leading to a very low LRBA expression in flow cytometry. He was started on IVIG replacement therapy and abatacept with a dose of 10 mg/kg per month. He experienced improvement in LP but continued to have CD. Meanwhile, Giardia antigen was detected in stool and did not respond to metronidazole but was successfully treated with nitrazoxanide. At the fourth month, the abatacept dose was increased to 20 mg/kg per month and later on to 20 mg/kg every 2 weeks, which resulted in complete resolution of diarrhea. Currently, he is at 33rd month of therapy without any adverse effects of abatacept except for mild eczematous lesions on his neck and seborrheic dermatitis on the scalp.

Patient P4 is a 13-year-old boy, brother of P2, who had recurrent LRTI and CD since early infancy. At the age of 9 years, he was admitted to the hospital because of persistent anal abscess and pancytopenia. The cytopenias resolved successfully with prednisolone therapy. He had cytomegalovirus infection and recently developed alopecia areata. Physical examination was notable for LP, which was similar to the clinical finding of his brother. He was evaluated for ID in the setting of a positive family history, and Sanger sequencing of *LRBA* identified the same frameshift mutation that was documented in P2 (c.7885delA, p.R2629fs). He was treated successfully with IVIG replacement and abatacept at a dose of 10 mg/kg per month. His alopecia resolved with topical steroids in addition to the abatacept, but recurrence was noted after 3 months. However, he had marked improvement of his LP and diarrhea after 6 months of therapy. He is still on abatacept for 33 months. Mild dermatophyte infections were recorded during abatacept without any other side effects.

Patient P5, a 27-year-old man, was a product of consanguineous parents and had CD since age 1 year. He also developed recurrent RTI, thrombocytopenia, splenomegaly, and palpable lymph nodes during follow-up. Immunoglobulin replacement was initiated at the age of 7 years. He developed arthritis in both knees, which resolved spontaneously. He received systemic steroids for cytopenias. The laboratory evaluation was compatible with lymphopenia, agammaglobulinemia, inverted CD4/CD8 T-cell ratio, low naive $CD4^+$ T cells, and low class-switched memory B cells. Combined immunodeficiency or common variable immunodeficiency (CVID) was considered. At the age of 20 years, he was admitted to the hospital with fever and vomiting and polypoid gastric mass was detected on abdominal computed tomography. Upper gastrointestinal endoscopy showed vegetative stomach tumor with a 4 × 4.5 cm diameter. The gastrectomy material showed grade I tubular adenocarcinoma. During follow-up, renal tubulopathy, massive splenomegaly, and relapsing CD were prominent. A short trial of methyl prednisolone, local budesonide, and sirolimus (2 mg/m^2/d) was beneficial in terms of controlling LP; however, sirolimus was stopped because of recurrent folliculitis complicating with skin abscess. Whole-exome sequencing showed a novel homozygous intronic variant in LRBA (c.767+5_767+8delGTAT, p?), confirmed later by Sanger sequencing. Abatacept was initiated at a 20 mg/kg dose every 2 weeks for LP, CD, and thrombocytopenia. After 9 months of therapy, a decision was taken for the patient to receive a transplant from his fully matched healthy sibling and currently he is doing well at the sixth month posttransplant, with 96% chimerism of donor cells.

Patient P6, an 11-year-old boy, was admitted to the clinic with recurrent LRTI and oral moniliasis since age 7 months. He had hepatosplenomegaly, clubbing, enlarged lymph nodes, and vitiligo, and the laboratory examination showed panhypogammaglobulinemia and low CD4, B, and NK cells. Antibiotic prophylaxis and immunoglobulin replacement were initiated. Chronic bloody and protein-losing diarrhea was documented since the age of 4 years, and pathological examination revealed lymphoid follicular hyperplasia, chronic inflammatory mucosa, and colitis with mild activation. During follow-up, he developed bilateral axillary and mediastinal lymph nodes. He had benefit from systemic corticosteroids, but the colitis relapsed during tapering. He also suffered from transient arthritis in both knees, which was resolved without specific therapy. Cytopenias were prominent since the age of 6 years as thrombocytopenia and anemia, requiring erythrocyte transfusion. At age 8 years, hydroxychloroquine was added to steroids for diarrhea and lymphoproliferative disease, but he could not tolerate the therapy because of the side effects. Whole-exome sequencing analysis showed a homozygous mutation in LRBA, c.2599C>T, p.Gln867*, and abatacept was initiated at a dose of 20 mg/kg monthly for LP, CD, and cytopenias. Although the respiratory symptoms ameliorated under 3 months of abatacept, his CD was not well controlled; therefore, HSCT was conducted. Unfortunately, he died after transplant at early stage because of graft versus host disease and sepsis.

Patient P7, a 4-year-old girl, was referred to the clinic because of early onset type 1 DM, presented at the age of 8 months with acute ketoacidosis. Her anti-glutamic acid decarboxylase and anti-insulin antibodies were positive and she was discharged with insulin replacement therapy. Molecular screening for early onset type 1 DM revealed a homozygous mutation in *LRBA* (c.5172-2A>G). Besides diabetes, she experienced mild to moderate bronchiolitis attacks. No lymphoproliferative disease and diarrhea were recorded. Immunoglobulin levels and lymphocyte subsets were in normal range. There was no LRBA expression on flow cytometry, and western blot analysis showed very faint expression of the protein. Monthly 20 mg/kg abatacept infusion was initiated when she was 1.5 years old. She is currently at the 25th month of abatacept and doing well except for poorly controlled diabetes.

Patient P8, a 15-year-old boy, presented at age 6 months with pneumonia. He subsequently developed chronic ITP, autoimmune hemolytic anemia (AIHA), CD, and LP, with cervical, axillary, and mediastinal lymphadenopathies and splenomegaly. He had low IgM and IgA levels and inverted CD4/CD8 T-cell ratio with low B cells and increased NK cells. He was positive for autoantibodies (antinuclear antibody, Coombs) and had elevated double-negative T cells. He had nodular lung disease. Genetic analysis revealed a mutation in LRBA (c.1963C>T, p.R655*). Because of the refractory cytopenias, he received an HSCT from his fully matched healthy sibling at the age of 10 years. After transplant, he suffered from grade IV acute graft versus host disease, which was controlled by systemic corticosteroids and cyclosporine.

Currently, he is doing well without symptoms and has 98% chimerism of donor cells.

Patient P9, a 21-year-old man, presented with severe diarrhea when he was 1.5 years old, followed by recurrent upper and lower RTIs. Since infancy, he has enlarged lymph nodes, splenomegaly, and pancytopenia. He developed Coombs-positive AIHA at age 16 years, but did not require immunosuppressive agents. He was also followed for transient arthritis in both knees. Physical examination was notable for growth failure, psoriasis-like rash, axillary and inguinal palpable lymph nodes, and hepatosplenomegaly. Leukopenia, thrombocytopenia, and low IgG and IgM levels were found on laboratory examination. The upper and lower gastrointestinal endoscopy showed diffuse nodular formation, which was described as glandular atrophy, eosinophilia in lamina propria, and germinal center hyperplasia. Thorax computed tomography (CT) showed bilateral multiple nodules and subcarinal lymph nodes. Flow cytometric analysis showed low LRBA expression and increased follicular T helper cells, consistent with LRBA deficiency. Sanger sequencing revealed c.2836_2839delGAAA, p.E946* mutation. During the follow-up, he relapsed with severe AIHA, which was partially controlled with high-dose steroids and cyclosporine. He had cushingoid appearance and severe osteoporosis; therefore, abatacept was initiated every week at a dose of 10 mg/kg and after 2 months of therapy, his LP, hemolytic anemia, and diarrhea were controlled without additional immunosuppressants. At the fifth month of abatacept therapy, he had a transplant from a fully matched unrelated donor. Currently, he is doing well at sixth month posttransplant with only mild diarrhea and has 98% chimerism of donor cells.

Patient P10 is a 35-year-old woman, a product of consanguineous marriage, who had multiple admissions due to recurrent sinopulmonary infections since childhood. She was diagnosed with Hashimoto disease at the age of 15 years and was started on L-thyroxine. At the age of 31 years, she was admitted because of bronchiectasis and atelectasis resulting from frequent lung infections (every month). She underwent a left lower lobectomy. She had CD for the last 2 years. She was found to have splenomegaly. Immunologic evaluation showed markedly low IgG, IgA, and IgM levels, no vaccine response, lymphopenia, and low CD4 and CD19 lymphocytes. Mediastinal multiple lymph nodes, cystic bronchiectasis in the upper lobe of the left lung, and diffuse frosted glass densities and consolidations in the right lung were detected. She was dependent on oxygen. Her upper gastrointestinal endoscopy was normal except for minimal mucosal hyperemia in the lower part of the esophagus. IVIG replacement therapy was started because of hypogammaglobinemia and related findings (especially pulmonary involvement). Mutation analysis detected a mutation in *LRBA* (c.7238dupG, p.S2413Rfs*1). Because of the CD, LP, and lower RTIs, 10 mg/kg abatacept was started every 4 weeks. After 6 doses of abatacept, her diarrhea and LP were partially controlled. Her spirometric examination revealed improved lung functions and she was off oxygen. The dose was adjusted to every 2 weeks, which resulted in full control of diarrhea.

Patient P11 is a 14-year-old boy, brother of P10, admitted because of recurrent lung infections and hepatosplenomegaly. At age 1 year, he was diagnosed with X-linked lymphoproliferative disorder because of LP and hemophagocytosis in the bone marrow. He also suffered from AIHA. Low IgG, IgA, and IgM levels and inverted CD4/CD8 ratio were noticed. Results for direct Coombs and anticardiolipin IgM were positive. He was started on regular IVIG therapy. Thorax CT showed bilateral ground glass opacity, multiple lymphadenopathies, atelectasis, and bronchiectasis. He was followed up with a diagnosis of lymphoproliferative disease and/or common variable immunodeficiency. Splenectomy was performed at the age of 11 years, because of persistent anemia. At the age of 12 years, he was diagnosed with hemophagocytic lymphohistiocytosis and chemotherapy was started according to hemophagocytic lymphohistiocytosis 2004 protocol. However, he died at the age of 14 years because of severe pulmonary infection. Twelve years later, when LRBA mutation was detected in his sister, genetic analysis was performed on his splenectomy material and the same *LRBA* mutation was detected.

Patient P12, a 23-year-old man, presented with fever and jaundice at age 3.5 years, which was diagnosed as AIHA and treated with long-term systemic corticosteroids. One year later, he also developed thrombocytopenia. At age 9 years, splenectomy was performed owing to persistent cytopenias. However, he continued to experience new episodes of hemolytic attacks, which require a high dose of corticosteroids. At the age of 10 years, CD was documented and biopsy findings were compatible with Crohn disease. He responded well to salazopyrin and oral steroids. When he was 17 years old, he was diagnosed with polyarticular juvenile idiopathic arthritis and responded to salazopyrin and adalimumab. Corticosteroid-induced severe osteoporosis was observed and required monthly systemic pamidronate disodium infusion. During the follow-up, he complained of unbalanced walking and blurred vision. Cranial magnetic resonance imaging demonstrated multiple plaques in his brain. He was evaluated for optic neuritis, transverse myelitis, and multiple sclerosis. Severe growth failure, hepatomegaly, axillary palpable lymph nodes, and clubbing were noted in his physical examination. Immunologic evaluation showed low IgG, IgA, and IgE levels, low $CD3^+$ T cells, inverted CD4/CD8 T-cell ratio, low class-switched B cells, and increased $CD21^{lo}38^{lo}$ B cells. Because of suspicion of an immunodysregulatory disease, flow cytometric analysis for LRBA protein was conducted and showed low expression with high follicular T cells compared with the control, mirroring LRBA deficiency. Whole-exome sequencing demonstrated *LRBA* mutation, confirmed by Sanger sequencing (c.2818dupC, p.Q940fs). Immunoglobulin replacement therapy and 15 mg/kg abatacept every other week were started. Complete resolution of diarrhea, arthritis, and LP was observed at the second month of therapy, although his neurologic symptoms continued. Currently, at 12th month of abatacept, he is off corticosteroids and other immunosuppressants such as salazopyrin and adalimumab.

Patient P13 is an 11-year-old boy referred to an immunology and allergy clinic for hypogammaglobulinemia and frequent RTIs. The patient had complaints of episodic nasal bleeding, skin bruising, abdominal pain, and cough. He was diagnosed with chronic ITP at age 6 years. His bone marrow examination was normal. He was undergoing steroid treatment for chronic ITP. In his follow-up, he was diagnosed with Evans syndrome due to hemolytic anemia. He had been hospitalized many times because of thrombocytopenia-induced bleeding attacks and he received intravenous steroid and occasional IVIG treatment. His parents' marriage was not consanguineous. His physical examination revealed decreased respiratory sounds, with crackles on the right lower lobe and hepatosplenomegaly. His laboratory investigations

showed panhypogammaglobulinemia, positive direct Coombs test result, and a very low C4 level. He had inverted CD4/CD8 ratio, low memory B cells, and high double-negative T cells (5.8%). The EBV-DNA and cytomegalovirus-DNA PCR were negative. The patient was diagnosed with autoimmune lymphoproliferative syndrome. He was started first on steroids, followed by MMF. Regular IVIG therapy and trimethoprim/sulfamethoxazole prophylaxis was started because of hypogammaglobulinemia. He got infections less frequently and the number of hospitalizations decreased. However, bleeding attacks due to hemolytic anemia and thrombocytopenia continued during the follow-up. Early onset autoimmunity, LP, and diarrhea were suggestive for LRBA deficiency, and mutation analysis showed a nonsense stop gained mutation (c.1963C>T, p.R655*). Because of CD and abdominal pain, a gastroduodenoscopy was performed, which revealed chronic atrophic gastritis and partial villous atrophy. The patient is now aged 18 years and is doing well on low-dose steroid and regular IVIG replacement.

Patient P14 is a 17-year-old girl who was admitted to the clinic with recurrent otitis, sinusitis, and sinopulmonary infection. Intractable diarrhea, nausea, and inadequate weight gain were notable in her medical history. She was diagnosed with celiac disease at the age of 13 months and was on a gluten-free diet between age 13 months and 6 years. After a gluten-free diet, her complaints improved partially. AIHA developed when she was 15 years old and was treated with corticosteroid. Meanwhile, regular IVIG therapy was started because of hypogammaglobulinemia and pneumonia. After IVIG therapy, her infections and AIHA were controlled, but diarrhea still continued. Her immunologic findings were remarkable for low IgA, IgM, and IgG levels, low B and memory B cells, and positive Coombs test result. Her lung CT demonstrated bilateral several parenchymal nodules and consolidation. The genetic analysis showed a mutation in *LRBA* (c.2735_2738delGGGT, p.Trp912*). Because of the uncontrolled CD and lung involvement, the patient was commenced on 10 mg/kg abatacept every 2 weeks, which resulted in resolution of diarrhea and better control of her symptoms after 2 months of therapy. Afterwards, the dose was switched to 15 mg/kg per month. Currently, she is on abatacept for 7 months without any side effects.

Patient P15, a 14-year-old boy, presented with recurrent RTI, hepatosplenomegaly, and generalized lymphadenopathies and experienced several episodes of Coombs-positive AIHA and immune thrombocytopenia. He was diagnosed with autoimmune lymphoproliferative syndrome due to high double-negative T cells (16%). During the follow-up, he developed interstitial lung disease and chronic hepatic disease with unknown etiology. Immunologic evaluation demonstrated low IgM level, low $CD19^+$B cell, low naive $CD4^+$ T cells, and high naive B-cell counts with decreased memory B cells. Immunosuppressive agents such as steroids (conventional and mega doses), MMF, cyclosporine A, and IVIG were used for intractable autoimmune cytopenias but were ineffective. He was splenectomized at age 10 years. At age 12 years, he was diagnosed with Hodgkin lymphoma due to the multiple lymphadenopathies detected in cervical and abdominal regions, which was controlled after treatment. Genetic analysis showed homozygous deletion in *LRBA* (c.3396-3397delAC, D975Yfs*15). Currently, he is on abatacept (10 mg/kg/wk) and doing well at second month of therapy.

Patients P16 (11 years) and P17 (12 years) are 2 male siblings born to consanguineous parents. Both had recurrent RTI, CD, failure to thrive, LP, and chronic glomerulonephritis. In addition, P16 had autoimmune thyroiditis and AIHA, whereas P17 had Evans syndrome and neutropenia. Because of lung infections in both patients, pulmonary findings such as bronchiectasis and lymphocytic interstitial pneumonia were observed during the follow-up. Immunologic evaluation of P16 showed normal IgG and IgM levels and a very low IgA level. The lymphocyte subsets were remarkable for low B cells and low naive $CD4^+$T cells. *Salmonella enteritidis* grew in stool culture of P16. In P17, all immunoglobulin levels were normal and the lymphocyte subsets were normal except for low NK cells. Both patients received sulfasalazine and corticosteroids for enteropathy. Regular antimicrobial therapy and IVIG replacement were started for both patients. During follow-up, P16 experienced numerous hospitalizations due to RTIs or exacerbation of enteropathy with hypoalbuminemia and electrolyte imbalances, which were treated with systemic corticosteroids. In P17, hemolytic anemia attacks were persistent despite oral corticosteroids; therefore, MMF was added to his therapy. The 2 affected patients with the same clinical phenotype were thought to have immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome or an immune dysregulation, polyendocrinopathy, enteropathy, X-linked−like syndrome. The genetic analysis revealed a nonsense mutation in *LRBA* (c.2496C>T, p.C832*). After molecular diagnosis, abatacept was started for both patients (10 mg/kg dose every 2 weeks). P16 had severe oral and esophageal candidiasis and fungal pneumonia (*Kloeckera apiculate* grew in pharyngeal swab) after the first dose of abatacept, which was not exactly directed to abatacept; however, abatacept was preferred to be stopped. MMF was added for autoimmune enteropathy, with only partial response. However, to control the CD, abatacept was restarted with the same dose, which was able to control patient's symptoms (diarrhea and failure to thrive) and allowed tapering the dose of corticosteroids and MMF. Currently, he is at 11th month of therapy. In P17, abatacept was initiated for Evans syndrome and interstitial lung disease. After 6 months of abatacept treatment, he has better clinical condition, with a short hospitalization for a mild LRTI. Currently, he is on abatacept at 18th month of therapy, with remission of Evans syndrome, diarrhea, and interstitial lung disease.

Patient P18, a 16-year-old girl, presented with recurrent LRTI at age 12 years. She was born to first cousin consanguineous parents. Her immunoglobulin levels were low, with IgG 320 mg/dL, IgA 25 mg/dL, and IgM 25 mg/dL, and she was evaluated for hypogammaglobulinemia/CVID diseases. Her vaccine responses were negative for hepatitis B and isohemagglutinin. Lymphocyte subset analysis showed low class-switched memory B cells. She was started on antibiotic prophylaxis and IVIG replacement. During follow-up, positive EBV PCR was detected without LP or hemophagocytic lymphohistiocytosis. Thorax CT scans showed bilateral chronic changes in lower lobes accompanied by bronchiectasis, atelectasis, and fibrosis. She was evaluated for genetic etiology related to CVID, and genetic analysis revealed a missense mutation in *LRBA* (c.5537C>T, p.S1846L). Abatacept was initiated at a dose of 10 mg/kg per 2 weeks. After 5 months of therapy, she has not experienced new infection episode or side effects related to abatacept.

Patient P19 (now aged 21 years) presented with recurrent RTIs since age 6 years, and she experienced ITP and autoimmune neutropenia. She recovered with high-dose IVIG, steroid, and azathioprine. At age 12 years, she was diagnosed with CVID

due to low serum immunoglobulins (IgA, 6 mg/dL; IgM, 117 mg/dL; and IgG, 459 mg/dL) and regular IVIG replacement therapy was initiated. After 2 years, she was diagnosed with AIHA and MMF was added to other immunosuppressants (steroid and azathioprine). During the follow-up, she started to lose weight and got CD. The endoscopic evaluation showed villus atrophy and celiac-like disorder. Gluten-free diet was started, which resulted in partial resolution of diarrhea. At age 19 years, AIHA relapsed, and an attempt was made to control it by corticosteroids. Genetic analysis revealed nonsense homozygous mutation in *LRBA* (c.7976C>G, p. S2659*). Abatacept was initiated at a 10-mg/kg dose every 2 weeks because of intractable diarrhea and hemolytic anemia, which was switched to a monthly therapy after 3 doses. Although hemolysis was controlled and diarrhea decreased over time, LP and inflammatory bowel disease did not respond well to abatacept. Currently, she is into her 20th month of therapy.

Patient P20 (now 5 years old) presented at age 6 months with CD, weight loss, and failure to thrive. She was initially diagnosed with non−IgE-mediated food allergy and was suspected to be suffering from immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome. She did not respond to extensive hydrolyzed formula and probiotics. At age 8 months, her weight loss and abdominal distension progressed. Celiac disease and lactose intolerance were considered, and a lactose-free diet was initiated without a clear benefit. At age 10 months, she developed bloody diarrhea complicated by generalize edema due to severe protein loss. Gastrointestinal biopsies revealed villous blunting and increased intraepithelial and lamina propria lymphocytes. He was diagnosed with celiac disease and started on a gluten-free diet without improvement. Later, the colonoscopic examination showed extensive colitis with eosinophilic infiltration. She was treated with corticosteroids, showing partial response. Her immunoglobulin levels and lymphocyte subset were normal; however, LRBA protein expression on flow cytometry was found to be low. Accordingly, LRBA mutation analysis showed a nonsense mutation in *LRBA* (c.1496C>A, S499*). IVIG replacement therapy and abatacept (10 mg/kg every 2 weeks) were initiated after diagnosis. After 3 doses of abatacept, she experienced total resolution of diarrhea with normal serum albumin level and was off steroids. Therefore, the dose of abatacept was extended to monthy infusion. Currently, she is doing well at the 21st month of therapy without any side effects.

Patients P21 and P22 (13 and 4 years old, respectively) were siblings and born to consanguineous parents. P21 was diagnosed with type 1 DM and followed with regular insulin injections since age 9 months. Between age 9 months and 5 years, her blood glucose levels were under good control. At age 6 years, she developed frequent diarrheal episodes and diagnosed with celiac-like disease and colitis. Corticosteroids and a gluten-free diet were started but revealed partial response. At age 8 years, arthritis developed in both knees, and was treated by corticosteroids. She was followed up with antibiotic prophylaxis and IVIG replacement therapy. Because of multiple autoimmune disorders including DM, celiac disease, colitis, and arthritis, genetic analysis was conducted. Whole-exome sequencing revealed homozygous frameshift insertion mutation in *LRBA* (c.3549_3550insA, p.A1184Sfs*34). Abatacept was started at a dose of 10 mg/kg every 2 weeks and after 3 doses was switched to a monthy therapy because of a stable disease course. Her CD was controlled and corticosteroid therapy was discontinued; however, arthritis partially responded to abatacept. The type 1 DM progressed and she was dependent on increased insulin injections. Therefore, she had a transplant from 9/10-matched unrelated donor at the 12th month of abatacept treatment. At 10th month posttransplant, she started to experience lung infections and CT imaging showed bronchiectasis, interstitial involvement, atelectasis, and fibrosis, which were thought to be bronchiolitis obliterans due to the chronic graft versus host disease. Her last chimerism was 98% of donor cells. Because of the recurrent infections and bronchiolitis obliterans, IVIG and cyclosporine were restarted. Currently, she is doing better without any new infection. P22 is a brother of P21 and he was asymptomatic and diagnosed after family screening for the LRBA mutation. After 2 years of follow-up, he started to experience frequent upper RTIs. Because of the severe phenotype of his sister, he was commenced on antibiotic prophylaxis and IVIG replacement therapy, which resulted in good control of infections.

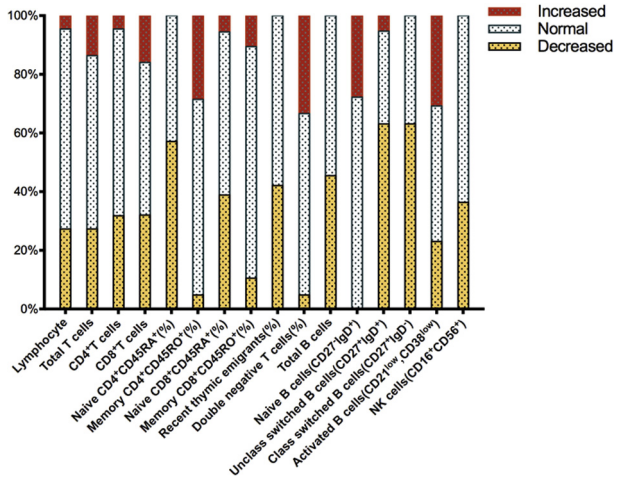

FIGURE E1. Flow cytometric analysis of peripheral blood in LRBA-deficient patients demonstrate abnormalities in T-, B-, and NK-cell compartments. Absolute numbers were provided for lymphocyte, total T, $CD4^+$, $CD8^+$ T, B, and NK cells, whereas the others were presented as percentage. Red bars with dots demonstrate increased cell numbers or percentage, white bars with dots show normal cell numbers or percentage, and yellow bars with dots display decreased cell numbers or percentage. The ranges are age-matched controls. Detailed results are provided in Table E2.

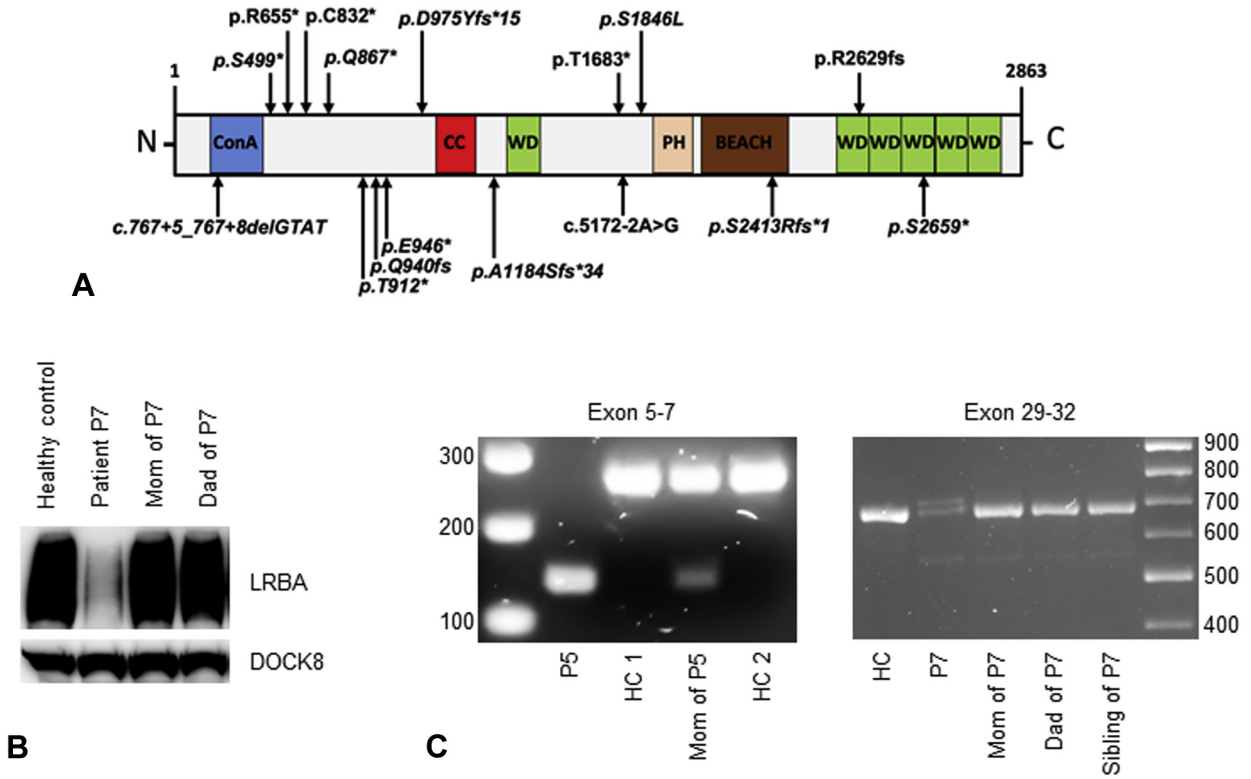

FIGURE E2. Mutations in LRBA deficiency are distributed throughout the gene. (**A**) Schematic diagram of LRBA protein domains, with locations of known and novel (italic) mutations observed in the cohort. Amino acid changes are presented by their single- letter code. Asterisk (*) indicates a premature stop codon. Two splice-site mutations (P5 and P7) were noted with complementary DNA levels. (**B**) Western blot analysis of LRBA protein in lymphocytes of patient P7 compared with parents and healthy control. Blots were reprobed for dedicator of cytokinesis 8 as a positive control. (**C**) cDNA analysis of the splice-site mutations in LRBA in P5 and P7 showed altered splicing. Primers were designed in the exons as indicated. (*Left*) Amplification of the cDNA with primers in exons 5 and 7 shows a truncated product for patient P5 as well as the mother (who is heterozygous for the splice-site deletion), indicating exon 6 skipping. Expected PCR product size was 261 bp and exon 6 was 122 bp. Sanger sequencing confirms the loss of exon 6 from patient P5. (*Right*) For P7, amplification of the cDNA with primers in exons 29 and 32 shows a truncated product as well as a slightly larger product than the expected size of 687 bp. Sanger sequencing of truncated band shows sequence for exon 30 and 32 but missing exon 31, indicating exon 31 skipping. Exon 31 is 134 bp, consistent with size of the truncation. Sanger sequencing of the upper bands shows partial retention of the intron (23 bp) 5′ of exon 31 in addition to the expected sequence of the exons (data not shown). *BEACH,* Beige/BEACH domain; *CC,* coiled coil; *Con A,* concanavalin A-like lectin domain; *DOCK8,* dedicatior of cytokinesis 8; *HC,* healthy control; *PH,* PH domain associated with beige/BEACH; *WD,* WD-40 repeat.

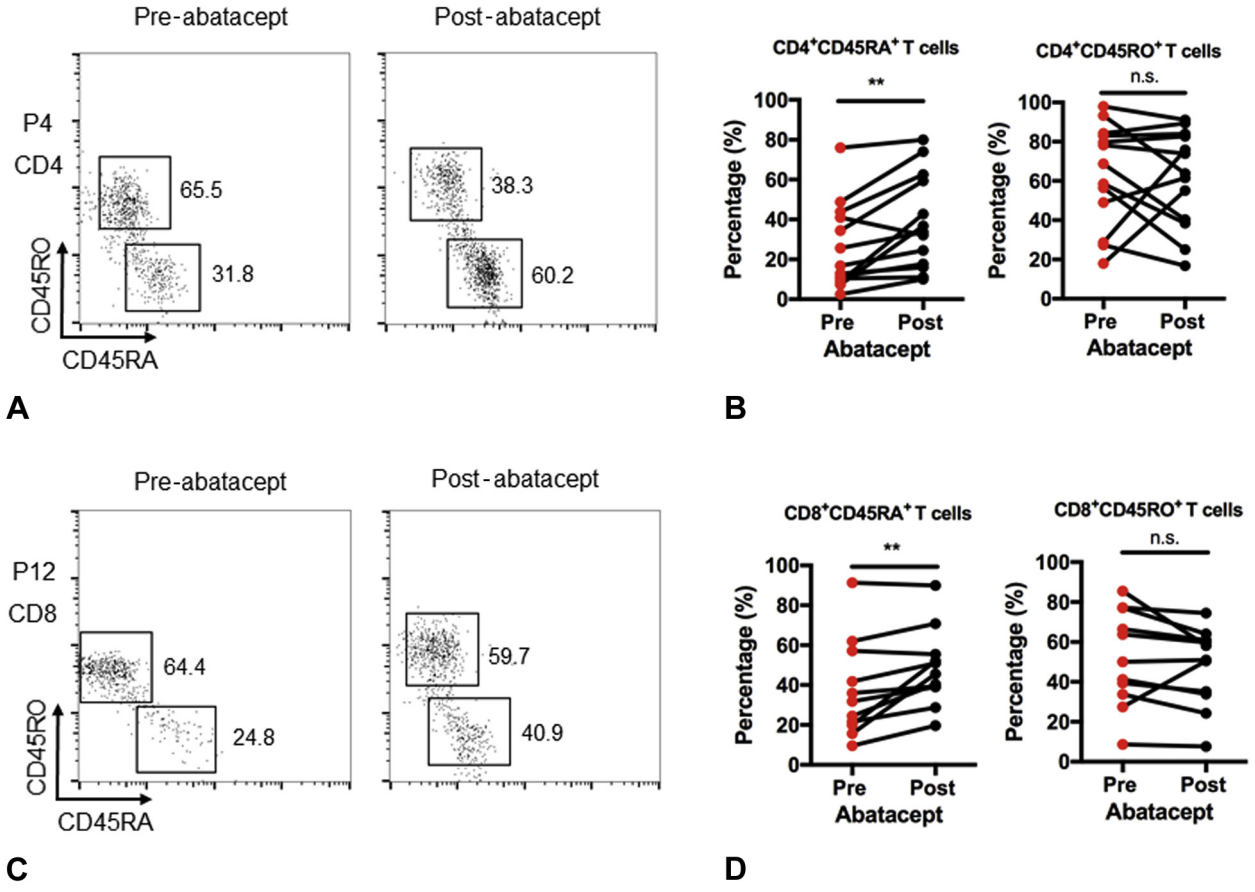

FIGURE E3. Increased frequency of naive T cells in LRBA-deficient patients with abatacept treatment. Flow cytometric analysis of circulating $CD4^+CD45RA^+/CD45RO^+$ (**A, B**) and $CD8^+CD45RA^+/CD45RO^+$ (**C, D**) T cells in LRBA-deficient patients before and after abatacept. *n.s.,* Not significant. $**P < .01$, Student unpaired and paired 2-tailed *t* test.

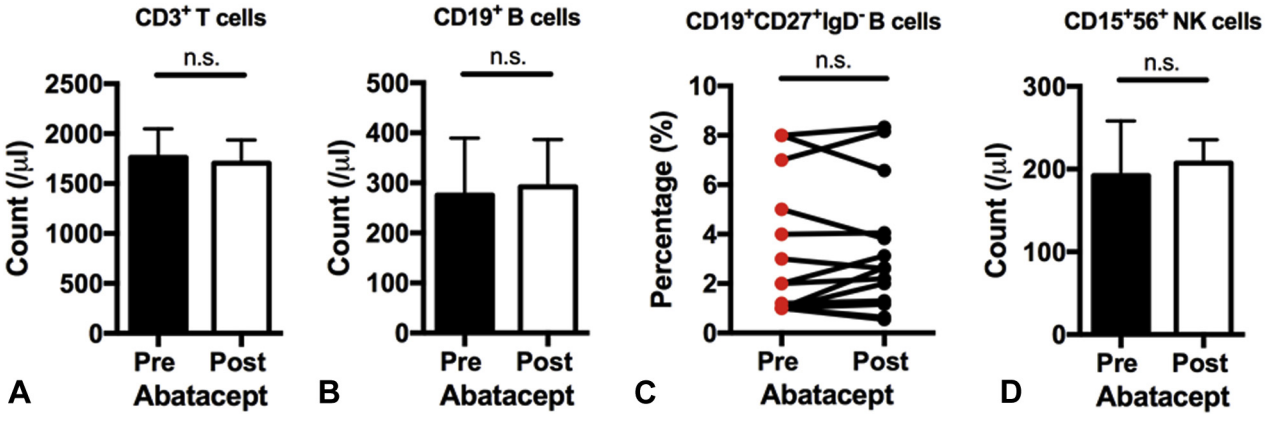

FIGURE E4. Comparisons of T-, B-, and NK-cell compartments before and after abatacept. Flow cytometric analysis of (**A**) circulating $CD3^+$ T cells, (**B**) $CD19^+$ B cells, (**C**) $CD19^+CD27^+IgD^-$ B cells, and (**D**) $CD16^+CD56^+$ NK cells. *n.s.*, Not significant. Student paired 2-tailed *t* test.

TABLE E1. The clinical findings and treatment options of LRBA-deficient patients

Parameters	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
Current age (mo)	156	113	73	153	312	136	35	171	219	422
Age of onset (mo)	18	48	8	1	3	7	8	6	18	84
Age at diagnosis (mo)	121	101	31	110	255	86	16	111	203	362
Recurrent infections										
Upper RTIs	✓	—	✓	✓	✓	✓	—	—	✓	✓
LRTIs	✓	—	—	—	✓	✓	✓	✓	✓	✓
Parenchymal lung disease	✓	—	—	—	✓	✓	✓	✓	✓	✓
Viral infections	✓	—	✓	✓	✓	✓	—	—	✓	—
Giardial infections	—	—	✓	—	—	—	—	—	—	—
Candidial infections	—	—	—	—	✓	✓	—	—	—	—
ID										
CD	✓	✓	✓		✓	✓	—	✓	✓	—
AIHA	—	—	—	—	—	—	—	✓	✓	—
ITP	✓	✓	—	✓	✓	✓	—	✓	—	—
T1DM	✓	—	—	—	—	—	✓	—	—	—
Alopecia	—	—	✓	—	—	—	—	—	—	—
Vitiligo	—	—	—	—	—	✓	—	—	✓	—
Arthritis	—	—	—	—	✓	✓	—	—	✓	—
Hashimoto thyroiditis	—	—	✓	✓	✓	—	—	—	—	✓
Optic neuritis	—	—	—	—	—	—	—	—	—	—
Demyelinating disease	—	—	—	—	—	—	—	—	—	—
LP										
Hepatomegaly	✓	✓		—	✓		—	—	✓	✓
Lymphadenopathy	✓	—	✓	✓	✓		—	✓	—	—
Splenomegaly	✓	✓	✓	✓	✓		—	—	✓	✓
Others										
Failure to thrive	✓	—	—	—	✓	—	—	✓	✓	✓
Skin abscess	—	—	—	✓	✓	✓	—	—	—	—
Clubbing	✓	—	—	—	—	✓	—	—	✓	—
Malignancy	—	—	—	—	✓	—	—	—	—	—
Osteoporosis	—	—	—	—	✓	—	—	—	✓	—
Pulmonary thromboembolism	—	—	—	—	✓	—	—	—	—	—
Cholecystitis	—	—	—	—	—	—	—	—	—	—
Chronic glomerulonephritis	—	—	—	—	—	—	—	—	✓	—
Deafness	—	—	—	—	—	—	—	—	—	✓
Medication										
Antimicrobial prophylaxis	✓	—	✓	✓	✓	✓	✓	✓	✓	—
Antifungal prophylaxis	✓	—	—	—	✓	—	—	—	—	—
Systemic corticosteroids	✓	✓	—	✓	✓	—	—	✓	✓	—
Budesonide	—	—	—	—	✓	—	—	—	—	—

MMF	—	✓	—	—	—	—	—	✓	—	—		
Sirolimus	✓	—	—	—	✓	—	—	—	—	—		
Cyclosporine A	—	✓	—	—	—	—	—	✓	✓	—		
Sulfasalazine	—	—	—	—	—	—	—	—	—	—		
Mesalazine	—	—	—	—	—	—	—	—	—	—		
Hydroxychloroquine	—	—	—	—	✓	—	—	—	—	—		
Abatacept	✓	✓	✓	✓	✓	✓	✓	—	✓	✓		
Adalimumab	—	—	—	—	—	—	—	—	—	—		
Immunoglobulin replacement	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		
Splenectomy	—	—	—	—	—	—	—	—	—	—		
HSCT	—	—	—	—	✓	✓	—	✓	✓	—		

Parameters	P11	P12	P13	P14	P15	P16	P17	P18	P19	P20	P21	P22
Current age (mo)	168	280	223	199	156	122	144	190	252	42	158	50
Age of onset (mo)	8	42	72	4	24	13	60	18	6	9	9	48
Age at diagnosis (mo)	108	279	129	176	58	37	96	168	84	10	123	32
Recurrent infections												
Upper RTIs	—	✓	✓	✓	—	—	✓	—	✓	—	—	✓
LRTIs	✓		✓	✓			✓		✓	—	—	—
Parenchymal lung disease	✓		✓	✓			✓		✓	—	—	—
Viral infections	✓		✓	—		—	✓		—	—	—	—
Giardial infections	—	—	—	—	—	—	—	—	—	—	—	—
Candidial infections	—	—	—	—	—	✓	—	—	✓	—	—	—
ID												
CD	—	✓	✓	✓	—	✓	✓	—	✓	✓	✓	—
AIHA	✓			✓	✓	—	✓	—	✓	—	—	—
ITP	—			—	✓	—	✓	—	✓	—	—	—
T1DM	—	—	—	—	—	—	—	—	—	—	✓	—
Alopecia	—	—	—	—	—	—	—	—	—	—	—	—
Vitiligo	—	—	—	—	—	—	—	—	—	—	—	—
Arthritis	—	✓	—	—	—	—	-	-	-	-	✓	—
Hashimoto thyroiditis	—	—	—	—	—	✓	—	—	—	—	—	—
Optic neuritis	—	✓	—	—	—	—	—	—	—	—	—	—
Demyelinating disease	—	✓	—	—	—	—	—	—	—	—	—	—
LP												
Hepatomegaly	✓	—	✓	—	✓	✓	✓	—	✓	—	—	—
Lymphadenopathy	✓	✓	—	—	✓	—	—	—	✓	—	—	—
Splenomegaly	✓	—	✓	—	✓	✓	✓	—	✓	—	—	—
Others												
Failure to thrive	—	✓	—	✓	✓	✓	✓	—	✓	✓	✓	—
Skin abscess	—	—	—	—	—	—	✓	—	—	—	—	—

(continued)

TABLE E1. *(Continued)*

Parameters	P11	P12	P13	P14	P15	P16	P17	P18	P19	P20	P21	P22
Clubbing	—	✓	—	—	—	—	✓	—	—	—	—	—
Malignancy	—	—	—	—	✓	—	—	—	—	—	—	—
Osteoporosis	—		✓	—	—	✓	✓	—	—	—	—	—
Pulmonary thromboembolism	—	—	—	—	—	—	—	—	—	—	—	—
Cholecystitis	—	—	—	—	—	—	—	—	—	—	—	—
Chronic glomerulonephritis	—	—	—	—	—	✓	✓	—	—	—	—	—
Deafness	—	—	—	✓	—	—	—	—		—	—	—
Medication												
Antimicrobial prophylaxis	—	✓	—	—	✓	✓	✓	✓	✓	✓		
Antifungal prophylaxis	—	—	—	—	—	✓	✓	—	—	—	✓	—
Systemic corticosteroids	—	✓	✓	—	✓	✓	✓	—	✓	—	—	—
Budesonide	—	—	—	—	—	—	—	—	—	—	—	—
MMF	—	—	—	—	✓	✓	✓	—	—	—	—	—
Sirolimus	—	—	—	—	—	—	—	—	—	—	—	—
Cyclosporine A	—	—	—	—	✓	—	—	—	—	—	—	—
Sulfasalazine	—	✓	—	—	—	—	—	—	—	—	—	—
Mesalazine	—	✓	—	—	—	—	—	—	—	—	—	—
Hydroxychloroquine	—	—	—	—	—	—	—	—	—	—	—	—
Abatacept	—	✓	—	✓	—	✓	✓	✓	✓	✓	✓	—
Adalimumab	—	✓	—	—	—	—	—	—	—	—	—	—
Immunoglobulin replacement	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
Splenectomy	✓	✓	—	—	✓	—	—	—	—	—	—	—
HSCT	—	—	—	—	—	—	—	—	—	—	✓	—

T1DM, Type 1 diabetes mellitus.

TABLE E2. The immunologic features of LRBA-deficient patients

	Patients (age at evaluation [y])					
Features	**P3 (4)**	**P7 (2.1)**	**P16 (3)**	**P20 (3.5)**	**P22 (3.8)**	**Reference value (2.01-≤5 y)**
Lymphocyte numbers (cells/μL)	1,100	5,200	3,200	5,400	2,000	2,128-7,820
Neutrophil numbers (cells/μL)	2,600	4,400	3,620	8,830	1,650	1,261-7,576
Hemoglobin (g/dL)	10	12.2	11.7	10.2	13	10-14
Platelets (cells/μL)	259,000	310,000	720,000	264,000	198,000	210.292-468.040
Immunoglobulins (mg/dL)						
IgG	2,370	605	664	636	971	604-1941
IgM	76	69	77	228	21	71-235
IgA	228	20	6.5	85.6	26	26-296
Immunophenotyping						
$CD3^+$cells (/μL) (%)	2,421	3,270	2,849	3,834	1,440	1,432.6-5,445.1
	(73.6)	(62.95)	(89)	(71)	(72)	57.2-82.7
$CD3^+CD4^+$ (/μL) (%)	1,217	2,012	1,888	1,944	840	722.2-3202.6
	(37)	(38.72)	(59)	(36)	(42)	25.3-55.5
$CD3^+CD8^+$ (/μL) (%)	950	1,087	768	1,566	500	387.6-2,303.9
	(28.8)	(20.91)	(24)	(29)	(26)	14-38.8
$CD3^+CD4^+CD45RA^+$ (/μL) (%)	527	1,520	712	ND	ND	446.3-2,445.9
	(43.9)	(76)	(41)	(68.6)	(62)	55.6-84.9
$CD3^+CD4^+CD45RO^+$ (/μL) (%)	703	546	925	ND	ND	212.6-783.3
	(58.6)	(27.3)	(49)	(23)	(30)	15.6-46.2
$CD3^+CD8^+CD45RA^+$ (/μL) (%)	374	986	ND	ND	ND	275-1,725.4
	(39.4)	(91.34)		(45.6)	(81.7)	46.4-100
$CD3^+CD8^+CD45RO^+$ (/μL) (%)	391	94	ND	ND	ND	62.7-615.5
	(41.2)	(8.66)		(1.72)	(12.4)	10-45.6
DNT cells (%)	4.79	1.97	0.39	ND	ND	1.1-3.5
RTE (/μL) (%)	480	1,453	299	ND	ND	389.8-2,285
	(40.5)	(72.66)	(39)	(38.5)	(38)	49-78
$CD19^+$ cells (/μL) (%)	552	1,407	38	1,134	280	322-1,033.4
	(16.87)	(27.05)	(1.2)	(21)	(14)	10.3-31.1
Naive $CD19^+CD27^-IgD^+$ (/μL) (%)	452	1,197	ND	ND	ND	191.8-1,429.7
	(82)	(85.51)		(84.9)	(93.4)	54.9-94.9
UCSM $CD19^+CD27^+IgD^+$ (/μL) (%)	58	117	ND	ND	ND	38.1-202.1
	(10.45)	(8.36)		(4.7)	(1.2)	6.3-23.1
CSM $CD19^+CD27^+IgD^-$ (/μL) (%)	21	65	ND	ND	ND	26.9-178.4
	(3.75)	(4.65)		(5.2)	(0.48)	2.9-31.9
$CD16^+/CD56^+$ cells (/μL) (%)	143	412	832	162	200	88.2-1,391.5
	(4.36)	(7.92)	(26)	(3)	(10)	2.5-29.4

Features	Patients (age at evaluation [y])					Reference value (5.01-≤10 y)	Patients (age at evaluation [y])				Reference values (10.01-≤16 y)
	P2 (8.2)	P4 (9)	P6 (8.5)	P11 (9.1)	P17 (7)		P1 (12)	P8 (10.9)	P15 (14)	P21 (12.2)	
Lymphocyte numbers (cells/μL)	800	1,100	2,000	13,600	1,380	1,801-5,635	2,300	3,800	7,400	2,500	1,490-4,104
Neutrophil numbers (cells/μL)	7,800	200	4,600	19,900	3,350	1,598-8,682	5,900	1,500	2,000	6,000	1,667-6,700
Hemoglobin (g/dL)	5	10.1	11.6	13.7	12.9	11-14	12.9	12.2	11.5	11.3	11-16
Platelets (cells/μL)	5,000	22,000	192,000	431,000	186,000	182.638-564.311	305,600	165,000	153,000	456,000	177.591-401.366
Immunoglobulins											
IgG (mg/dL)	754	910	247	649	1,020	745-1,804	505	1,580	765	1,390	987-1,958
IgM (mg/dL)	101	141	8	21.3	226	78-261	19	47	47	939	83-282
IgA (mg/dL)	24.8	47	47	<6	62	57-282	25	27	90	297	96-465
Immunophenotyping											
$CD3^+$cells (/μL) (%)	672	775	1,860	1,171	1,048	1,213.7-4,128.1	1,615	2,600	6,779	1,525	1,033.3-3,305.4
	(84)	(70.48)	(93.41)	(86.1)	(76)	55.7-88.6	(70.2)	(68.42)	(91.61)	(61)	58.5-87.9
$CD3^+CD4^+$ (/μL) (%)	440	495	374	5,005	510	607.4-2,111.8	1,035	1,058	2,589	900	504.1-1,777.1
	(55)	(45.06)	(18.71)	(36.8)	(37)	24.8-50.9	(45)	(27.85)	(34.98)	(36)	28.2-47.7
$CD3^+CD8^+$ (/μL) (%)	224	248	1,280	6,065	445	379.4-2,083.1	695	1,316	3,495	625	381.1-1,312.5
	(55)	(22.53)	(64.13)	(44.6)	(33.2)	17.5-42.6	(27.8)	(34.62)	(47.23)	(25)	17.6-42.8
$CD3^+CD4^+CD45RA^+$ (/μL) (%)	ND	ND	344	ND	ND	312.9-1,432.9	77	36	265	ND	181.8-1,202.6
		(34.5)	(91.97)			42.9-81.5	(7.45)	(8.77)	(10.24)	(25.1)	36.1-74.1
$CD3^+CD4^+CD45RO^+$ (/μL) (%)	ND	ND	275	ND	ND	256.5-786.3	966	405	2,185	ND	239.8-826.2
		(68.7)	(73.64)			26.8-58.7	(93.3)	(97.61)	(84.41)	(62.5)	30.9-71.7
$CD3^+CD8^+CD45RA^+$ (/μL) (%)	ND	ND	1,168	ND	ND	208.6-1,392.8	109	667	2,377	ND	206.9-701.1
		(62)	(91.28)			39.1-93.9	(15.7)	(43.32)	(68)	(21.5)	32.9-93.7
$CD3^+CD8^+CD45RO^+$ (/μL) (%)	ND	ND	374	ND	ND	74.2-921.2	595	1,121	1,102	ND	107.9-606.3
		(33.8)	(29.21)			11.1-77.9	(85.5)	(72.8)	(31.54)	(48.6)	17.9-72.8
DNT cells (%)	2.5	4.43	2.96	ND	1.2	0.9-5.5	2.38	2.73	2.6	ND	0.8-2.5
RTE (/μL) (%)	ND	ND	19	ND	488	258-1,342.4	67	62	107	ND	175.1-969.9
		(32.4)	(5.07)		(37)	37-73	(6.47)	(15)	(4.12)	ND	28-67
$CD19^+$ cells (/μL) (%)	72	201	27	1,292	289	197.1-867.3	288	38	81	375	94-792.8
	(9)	(18.31)	(1.33)	(9.49)	(21)	7.4-22.8	(12.5)	(1)	(1.09)	(15)	5.2-21.8
Naive $CD19^+CD27^-IgD^+$ (/μL) (%)	ND	145	22	ND	ND	108.3-646.9	264	252	76	ND	51-614.8
		(72.36)	(81.48)			5.5-90.2	(91.5)	(72.33)	(94.12)	(96)	45.7-92.1
UCSM $CD19^+CD27^+IgD^+$ (/μL) (%)	ND	44	4	ND	187	18.3-159.1	12	30	1	ND	9.1-107
		(21.9)	(14.81)		(65)	5.9-28.8	(4.2)	(8.63)	(1.47)	(0.4)	4.7-28
CSM $CD19^+CD27^+IgD^-$ (/μL) (%)	ND	5	0	ND	28	24.2-147.4	3.3	51	0	ND	13.7-117.4
		(2.5)	(0)		(10)	6.7-31.1	(1.16)	(14.68)	(0)		6.1-35.2
$CD16^+/CD56^+$ cells (/μL) (%)	56	113	66	382	27	111.3-963.8	184	1,149	1,052	275	94.4-1,774.8
	(7)	(10.23)	(3.32)	(2.81)	(2)	4-29	(8)	(30.23)	(14.21)	(11)	4.7-35.2

Features	Patients (age at evaluation [y])								Reference value (>16 y)
	P5 (23)	P9 (19)	P10 (35)	P12 (23)	P13 (16)	P14 (17)	P18 (16)	P19 (21)	
Lymphocyte numbers (cells/μL)	600	800	810	4,500	2,530	2,206	3,300	600	1,344-3,817
Neutrophil numbers (cells/μL)	3,600	1,700	4,170	9,900	9,900	5,910	9,710	6,700	2,068-8,533
Hemoglobin (g/dL)	11.3	11.2	10.9	14.2	13.2	12.5	13.1	12.7	11-16
Platelets (cells/μL)	142,000	91,000	135,000	375,000	5,000	155,000	219,000	125,000	165.726-382.709
Immunoglobulins (mg/dL)									
IgG	407	527	<134	748	570	137	320	459	913-1,884
IgM	50	30	<15.6	210	53	17	25	1,174	88-322
IgA	25	80	5	15	48	25	25	6	139-378
Immunophenotyping									
$CD3^+$cells (/μL) (%)	514	600	705	3,652	2,150	1,800	2,842	521	1,024.4-2,793.5
	(85.6)	(74.9)	(88.07)	(81.16)	(85)	(81.61)	(86.12)	(86.79)	65.0-85.7
$CD3^+CD4^+$ (/μL) (%)	240	344	211	1,485	986	732	1,482	326	621.0-1,631.7
	(39.99)	(43.4)	(26.38)	(33)	(39)	(33.18)	(44.91)	(54.26)	33-59.5
$CD3^+CD8^+$ (/μL) (%)	266	224	469	2,055	1,163	1,050	1,270	182	269.9-1,255.1
	(44.29)	(27.8)	(58.6)	(45.67)	(46)	(47.59)	(38.47)	(30.32)	16-41.8
$CD3^+CD4^+CD45RA^+$ (/μL) (%)	ND	58	22	381	636	313	724	42	129.9-1,177.9
	(2.5)	(16.8)	(10.43)	(25.63)	(21)	(42.75)	(48.85)	(12.85)	14-99.8
$CD3^+CD4^+CD45RO^+$ (/μL) (%)	ND	276	178	1,163	2,242	403	837	270	2,78.9-1,138.1
	(98)	(79.7)	(84.24)	(78.3)	(74)	(55.07)	(56.46)	(82.97)	33.8-92.4
$CD3^+CD8^+CD45RA^+$ (/μL) (%)	ND	28	45	503	1,393	528	531	58	107-793.1
	(21.6)	(57.1)	(9.55)	(24.5)	(46)	(50.33)	(41.78)	(31.84)	25.9-98.4
$CD3^+CD8^+CD45RO^+$ (/μL) (%)	ND	88	362	1,312	1,484	530	636	121	68.8-745.6
	(77.1)	(39.4)	(77.21)	(63.86)	(49)	(50.52)	(50.09)	(66.46)	13.9-100
DNT cells (%)	1.45	2.1	1.88	9.71	9	2.68	3.41	1.48	0.5-3.9
RTE (/μL) (%)	ND	39	16	281	1,340	209	549	28	59.6-1,306.5
	(1.5)	(17.4)	(7.6)	(18.92)	(53)	(28.53)	(37.04)	(8.66)	7-100
$CD19^+$ cells (/μL) (%)	26	68	4	348	177	16	196	24	87.2-540.3
	(4.29)	(8.5)	(0.47)	(7.73)	(7)	(0.72)	(5.93)	(3.95)	4.7-18
Naive $CD19^+CD27^-IgD^+$ (/μL) (%)	ND	61	2	177	2,403	14	112	13	47.6-304.2
	(78.1)	(89.3)	(57.69)	(50.92)	(95)	(87.5)	(57.2)	(55.67)	36.2-85
UCSM $CD19^+CD27^+IgD^+$ (/μL) (%)	ND	4	1	11	50	0	2	0	11.8-110.5
	(6.6)	(5.5)	(15.38)	(3.07)	(2)	(0)	(1.14)	(0)	7.9-38.2
CSM $CD19^+CD27^+IgD^-$ (/μL) (%)	ND	2	0	28	25	1	5	0.87	10.3-148.8
	(1.8)	(1)	(9.62)	(7.99)	(1)	(8.33)	(2.65)	(1)	8.2-44
$CD16^+/CD56^+$ cells (/μL) (%)	45	88	60	88	212	256	201	42	100-640.1
	(7.48)	(11.1)	(7.54)	(1.95)	(7)	(11.6)	(6.09)	(7.05)	5.1-24.6

CSM, Class-switched memory; *DNT,* double-negative T; *ND,* not determined; *RTE,* recent thymic emigrants; *UCSM,* unclass-switched memory.

TABLE E3. The immunologic features of patients according to the cT_{FH} cells

Features	Patients with persistent high cT_{FH} after abatacept (n = 2)	Patients with decreased cT_{FH} after abatacept (n = 7)	*P* value
Age at the evaluation (y)	14.1 ± 10.3	15.7 ± 9.2	NS
Time of follow-up (y)	4.2 ± 2.1	2.1 ± 1.1	.04
Immunophenotyping			
Lymphocyte (cells/μL)	807 ± 395	2793 ± 1781	.03
$CD3^+$ cells (cells/μL)	580 ± 108	2296 ± 1155	.03
$CD19^+$ cells (cells/μL)	25 ± 1.4	387 ± 515	.03
$CD16^+/CD56^+$ cells (cells/μL)	43 ± 9.6	184 ± 121	.02
$CD3^+CD4^+CD45RA^+$ (%)	8.5 ± 5.4	36.9 ± 21.2	NS
$CD3^+CD4^+CD45RO^+$ (%)	90.3 ± 7.5	64.6 ± 20.1	NS
CSM $CD19^+CD27^+IgD^-$ (%)	1.1 ± 1.0	4.4 ± 3.0	NS

CSM, Class-switched memory; *NS,* not significant.
Values are mean ± SD.