Journal of Clinical Immunology (2019) 39:726–738
https://doi.org/10.1007/s10875-019-00677-6

ORIGINAL ARTICLE

A Spectrum of Clinical Findings from ALPS to CVID: Several Novel *LRBA* Defects

Deniz Cagdas[1] · Sevil Oskay Halaçlı[2] · Çağman Tan[2] · Bernice Lo[3] · Pınar Gür Çetinkaya[1] · Saliha Esenboğa[1] · Betül Karaatmaca[1] · Helen Matthews[4] · Burcu Balcı-Hayta[5] · Tuba Arıkoğlu[6] · Fatih Ezgü[7] · Elifcan Aladağ[8] · İnci N. Saltık-Temizel[9] · Hülya Demir[9] · Barış Kuşkonmaz[10] · Visal Okur[10] · Fatma Gümrük[10] · Hakan Göker[8] · Duygu Çetinkaya[10] · Kaan Boztuğ[11] · Michael Lenardo[4] · Özden Sanal[1] · İlhan Tezcan[1]

Received: 5 April 2019 / Accepted: 25 July 2019 / Published online: 20 August 2019

Abstract

Introduction Autosomal recessively inherited lipopolysaccharide-responsive beige-like anchor (*LRBA*) protein deficiency was shown to be responsible for different types of inborn errors of immunity, such as common variable immunodeficiency (CVID) and autoimmune lymphoproliferative syndrome (ALPS). The aim of this study was to compare patients with LRBA-related ALPS and LRBA-related CVID, to describe their clinical and laboratory phenotypes, and to prepare an algorithm for their diagnosis and management.

Methods Fifteen LRBA-deficient patients were identified among 31 CVID and 14 possible ALPS patients with Western blotting (WB), primary immunodeficiency disease (PIDD) gene, next-generation panel screening (NGS), and whole exome sequencing (WES).

Results The median age on admission and age of diagnosis were 7 years (0.3–16.5) and 11 years (5–44), respectively. Splenomegaly was seen in 93.3% (14/15) of the patients on admission. Splenectomy was performed to 1/5. Recurrent upper respiratory tract infections (93.3% (14/15)), autoimmune cytopenia (80% (12/15)), chronic diarrhea (53.3% (8/15)), lower respiratory tract infections (53.3% (8/15)), lymphoma (26.6% (4/15)), Evans syndrome (26.6% (4/15)), and autoimmune thyroiditis (20% (3/15)) were common clinical findings and diseases. Lymphopenia (5/15), intermittant neutropenia (4/15), eosinophilia (4/15), and progressive hypogammaglobulinemia are recorded in given number of patients. Double negative T cells ($TCR\alpha\beta^{+}CD4^{-}CD8^{-}$) were increased in 80% (8/10) of the patients. B cell percentage/numbers were low in 60% (9/15) of the patients on admission. Decreased switched memory B cells, decreased naive and recent thymic emigrant (RTE) Thelper (Th) cells, markedly increased effector memory/effector memory RA^{+} (TEMRA) Th were documented. Large $PD1^{+}$ population, increased memory, and enlarged follicular helper T cell population in the $CD4^{+}$ T cell compartment was seen in one of the

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10875-019-00677-6) contains supplementary material, which is available to authorized users.

✉ Deniz Cagdas
deniz.ayvaz@hacettepe.edu.tr

[1] Department of Pediatrics, Division of Pediatric Immunology, Hacettepe University Medical School, Ankara, Turkey

[2] Institute of Child Health, Immunology, Hacettepe University, Ankara, Turkey

[3] Sidra Medical and Research Center, Al Rayyan, Qatar

[4] National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, USA

[5] Department of Medical Biology, Hacettepe University Medical School, Ankara, Turkey

[6] Department of Pediatrics, Division of Allergy and Immunology, Mersin University Medical School, Mersin, Turkey

[7] Department of Pediatrics, Division of Pediatric Inborn Metabolic Disorders, Metabolism and Genetics, Gazi University Medical School, Ankara, Turkey

[8] Department of Internal Medicine, Division of Hematology, Hacettepe University Medical School, Ankara, Turkey

[9] Department of Pediatrics, Division of Pediatric Gastroenterology, Hacettepe University Medical School, Ankara, Turkey

[10] Department of Pediatrics, Division of Pediatric Hematology, Hacettepe University Medical School, Ankara, Turkey

[11] CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria

patients. Most of the deleterious missense mutations were located in the DUF1088 and BEACH domains. Interestingly, one of the two siblings with the same homozygous LRBA defect did not have any clinical symptom. Hematopoietic stem cell transplantation (HSCT) was performed to 7/15 (46.6%) of the patients. Transplanted patients are alive and well after a median of 2 years (1–3). In total, one patient died from sepsis during adulthood before HSCT.

Conclusion Patients with LRBA deficiency may initially be diagnosed as CVID or ALPS in the clinical practice. Progressive decrease in B cells as well as IgG in ALPS-like patients and addition of IBD symptoms in the follow-up should raise the suspicion for LRBA deficiency. Decreased switched memory B cells, decreased naive and recent thymic emigrant (RTE) Th cells, and markedly increased effector memory/effector memory RA^+ Th cells (TEMRA Th) cells are important for the diagnosis of the patients in addition to clinical features. Analysis of protein by either WB or flow cytometry is required when the clinicians come across especially with missense LRBA variants of uncertain significance. High rate of malignancy shows the regulatory T cell's important role of immune surveillance. HSCT is curative and succesful in patients with HLA-matched family donor.

Keywords LRBA deficiency · LATAIE · Hsct · Malignancy

Introduction

Primary immunodeficiencies are an extremely heterogeneous group of disorders generally inherited by Mendelian pattern. Lipopolysaccharide-responsive beige-like anchor protein (*LRBA*) deficiency was seperately discovered by two different groups in 2012 [1, 2]. According to the recent reports, *LRBA* defects have been suggested to be one of the most common autosomal recessive defects causing common variable immunodeficiency (CVID) [3], and it is also shown to be responsible from autoimmune lymphoproliferative syndrome (ALPS)-like (ALPS-like) disease [4].

LRBA molecule has a role in regulating cell surface expression of CTLA-4 [5]. As CTLA-4 is a checkpoint inhibitor of T cell function, LRBA deficiency is associated especially with the loss of regulatory T cell (Treg) function. Autoimmune cytopenia and early-onset and persistant diarrhea are among the leading manifestations [1, 6, 7]. It causes a highly variable disease, "LRBA deficiency with autoantibodies, Treg defects, autoimmune infiltration, and enteropathy" (LATAIE) disease [5].

Immunologic abnormalities reported in LRBA-deficient patients have included deficient T cell activation/proliferation, increased circulating follicular helper T cells [8], defects in specific antibody response, decreased IgG antibody production, decreased autophagy, and increased apoptosis in B lymphocytes [2]. The majority of LRBA-deficient patients have low switched memory B cells and plasmablasts [2, 9].

As ALPS-like or CVID-like presentation is commonly seen in patients with LRBA deficiency, the aim was to evaluate and compare patients with LRBA-ALPS and LRBA-CVID and prepare an algorithm to ease the diagnosis and follow-up of the patients with LRBA. We performed LRBA gene sequencing and protein studies in the patients with "probable ALPS" and CVID.

Patients and Methods

Patients

Fifteen LRBA-deficient patients from 14 families diagnosed and followed up with the diagnosis of CVID and possible ALPS between years 2012 and 2019 in Hacettepe University Immunology unit were enrolled into the study. One homozygous LRBA defective 16-year-old female patient was excluded as *LRBA* and *CTLA-4* expressions were found to be normal in FACS analysis.

The patients were defined in cohorts of CVID (Project number GO13/228 and GO15/370) and ALPS (probable ALPS) (Project number GO13/265 and GO11/19-23) patients.

The criteria of probable ALPS in the study of Oliviera et al. were fulfilled in for the patients with ALPS [10].

Methods

Flow Cytometry

Lymphocytes The analysis of peripheral blood lymphocyte populations was performed by 6-color flow cytometry (Attune NxT, Thermo Fisher, USA), using 100 μl of whole blood stained with 20 μl of monoclonal antibodies with fluorescein isothiocyanate (FITC), phycoerythrin (PE), allophycocyanin (APC), or peridinin–chlorophyl–protein (PerCP)) against T and B subset markers (obtained from eBioscience, Thermo Fisher, USA) and incubated in the dark for 15 min at room temperature. For lymphocyte analysis, CD3(FITC), CD4(FITC), CD8(PE), $CD16^+56$(PE), and CD19(PE) were used and for T and B lymphocyte subgroup analysis, CD4(FITC), CCR7(PE), CD31(PE), CD45RA (APC), and CD8(PerCP) (Beckton Dickinson, BD, USA) were used.

Flow Cytometric Analysis of LRBA Protein

PBMCs or activated T cells were fixed, permeabilized, and stained as previously described [11]. In brief, cells were fixed and permeabilized using eBioscience Foxp3 staining kit, stained with anti-LRBA (Sigma) antibodies followed by anti-rabbit fluorophore conjugated secondaries, and then analyzed by flow cytometry.

Flow Cytometric Analysis of Tfh and Treg Cells

This analysis [5] was performed for two (P8, PX) of the patients. PBMCs were surface stained for CXCR5 and PD-1, then washed, fixed, and permeabilized using eBioscience Foxp3 staining kit. Cells were then stained with antibodies for CD4, CTLA-4, CD45RA, and FoxP3. Tfh cells were gated as $CD4^+CXCR5^+PD\text{-}1^+$. Treg were gated as $CD4^+Foxp3^+$ and assessed for CTLA-4 expression. Fixed and permeabilized PBMCs were also stained for CD4, CD25, and FoxP3 to assess CD25 expression on Tregs.

CTLA-4 Level Analysis Total CTLA-4 levels were assessed in $CD4^+Foxp3^+$T cells by intracellular staining.

Western Blot Analysis Some patients are analyzed first for presence of LRBA protein. Mononuclear cells were isolated from peripheral blood using density gradient centrifugation (Capricorn Scientific, Germany). The cells lysed using 1× cell lysis buffer (Cell Signaling Technologies, USA) including 1 mM phenylmethylsulphonyl fluoride and centrifuged 13.000 rpm at 4 °C for 10 min. Protein samples were separated using 6% stacking and 9% resolving gel at 150 V for 1 h. The separated protein samples were tranferred to PVDF (polyinylidene difluoride) membrane using wet transfer system (Promega, USA) at 230 V for 3 h in chilled conditions. Following transfer, membrane was blocked with 5% skimmed milk in phosphate buffer saline including 1% Tween-20 (Sigma-Aldrich, USA). Protein bands were treated with WesternBright ECL (Advansta, UK) and visualized by GeneGnome XRQ (Syngene, UK).

Rabbit LRBA antibody (#HPA023597) was from Sigma-Aldrich (USA), and anti-rabbit secondary antibody (#7074) was from Cell Signaling Technologies (USA).

Genetic Analysis

Whole Exome Sequencing WES was performed for 8 patients (P1, 2, 3, 5, 6, 7, 9) [12].

Targeted PID Panel Screening HaloPlexTM probes which were designed to capture 356 PID-related genes (P4, 11, 14) were used as described in the reference [13].

A few CVID patients (P8, 10, 13, 15) were diagnosed by screening another PID gene panel [14].

All patients were Sanger sequenced after NGS-based gene panel screening.

Sanger Sequencing Genomic DNA was extracted from 200 μl of blood using the QIamp DNA Blood Kit (Qiagen), according to the manufacturer's instructions. PCR reactions were carried out in 100 μl reaction volume containing 50 ng of DNA, buffer 10X, 1.5 mM MgCl2, 0.2 mM dNTPs, 2.5 U AmpliTaq Gold (Applied Biosystems), and 50 pmol of primers designed to amplify all LRBA coding exons (Supplementary Table 1). PCR conditions were as follows: a denaturation step at 95 °C for 5 min; 5 cycles 35 cycles at 95 °C for 1 min, 58 °C for 1 min and 72 °C for 1 min, and a final extension step at 72 °C for 10 min. PCR products were directly sequenced using the appropriate primers and BigDye® Terminator v3.1 Cycle Sequencing Kits (Applied Biosystems). The products were loaded on a 3130 Genetic Analyzer, and the results were analyzed with Sequencing Analysis v.5.2 software.

Results

Patients' Characteristics

There were 15 patients from 13 families (Table 1). Female/male ratio was 6/9, and parental consanguinity ratio was 14/15 (92.86%). The median age on admission was 7 years (0.3–16.5). The median age of diagnosis was 11 years (5–44). There was no difference between the age on admission of patients with LRBA-ALPS and LRBA-CVID ($p > 0.05$).

Clinical and laboratory characteristics of the patients are described in Tables 1, 2, 3, and 4.

Seven patients (P1, 2, 4, 5, 6, 9, 14) (50%) were diagnosed among the group of 14 patients with ALPS (Fig. 1). They all fit "probable ALPS" criteria [10] (Table 2); however, no *FAS*, *FASL*, and *caspase 10* mutations were found [19].

P3 and P7 were sisters of P2 and P6 respectively.

Eight patients (P3, 7, 8, 10, 11, 12, 13, 15) (25.8%) were diagnosed among the group of 31 patients with CVID presenting with autoimmune diseases, inflammatory bowel disease, and lymphoproliferation.

Diagnostic Characteristics WES is needed in 5/7 (71.4%) of ALPS-LRBA and 1/7 (14.3%) of CVID-LRBA patients.

Clinical Characteristics

Infectious Diseases Recurrent upper (14/15, 93.3%) and lower (8/15, 53.3%) respiratory infections were common in the patients. CMV pneumonia was seen in P12 and P14.

Table 1 Clinical features of the patients with LRBA Deficiency

Patients	Age(year)/ gender	Age at clinical presentation (year)	Age at diagnosis	Consanguinity/ family history	Infections	Autoimmune/ inflammatory disease	Lymphoproliferation/ malignity	Immunomodulatory therapy	Other therapies
P1	6.5/M	4	5	No	Recurrent URTI, pneumonia	ITP	Splenomegaly	Steroids, cyclosporin a, abatacept	IVIG
P2 [15]	9/M	4.5	7	Yes	Recurrent URTI	AHA, ITP, thyroiditis, IBD-like colitis	Splenomegaly	Steroids, cyclosporin a, MMF, abatacept	HSCT
P3 [15]	17.5/F	16.5	15	Yes	No	No	No	No	No
P4 [16]	15/M	6	13	Yes	Recurrent URTI, fungal infections, pneumonia	AHA, thyroiditis, IBD-like colitis	Splenomegaly	Steroids, cyclosporin a, MMF, abatacept	IVIG, HSCT
P5	11/M	4.5	9	Yes	URTI	AHA, ITP	Splenomegaly, Hodgkin lymphoma (lymphocyte-rich type)	Steroids, cyclosporin a, MMF, abatacept	IVIG, chemotherapy
P6 [16]	17.5/F	11	14	Yes	URTI	ITP	Splenomegaly	Steroids, cyclosporin a, MMF	IVIG, HSCT
P7	15/M	12.5	After death	Yes	URTI	AHA, ITP	Splenomegaly	Steroids, cyclosporin a	IVIG
P8	26/M	5	26	Yes	Recurrent URTI, pneumonia, BK viremia	ITP, hypersplenism), celiac disease, coronary vasculitis	Splenomegaly, non-Hodgkin lymphoma (B cell lymphoma)	Steroids, cyclosporin a, MMF, abatacept	IVIG, chemotherapy, splenectomy, HSCT
P9	19/M	9	10	Yes	Recurrent URTI	Trombocytopenia, leukopenia (hypersplenism), Raynaud phenomena, celiac disease	Gastric Ca, splenomegaly	Steroids, cyclosporin a, MMF, abatacept	IVIG, chemotherapy, HSCT
P10	11/M	5	7	Yes	Recurrent URTI, pneumonia (*Aspergillus*)	ITP	Non-Hodgkin lymphoma (follicular lymphoma)	Abatacept	IVIG, chemotherapy
P11	9/F	1	6	Yes	Recurrent URTI pneumonia, diarrhea	ITP, AHA, IBD-like colitis	Splenomegaly	Steroids, cyclosporin a, MMF, abatacept	IVIG
P12 [16]	18/M	1	11	Yes	Recurrent URTI pneumonia, CMV infection	IBD-like colitis	Splenomegaly	Steroids, ASA, cyclosporin a, MMF, abatacept	IVIG, HSCT
P13	32/F	16	32	Yes	Recurrent URTI, pneumonia, CMV infection, recurrent herpes	IBD-like colitis, Crohn's disease	Splenomegaly, non-Hodgkin lymphoma (B cell)	Steroids, ASA	Splenectomy,IVIG
P14	11/F	7	11	Yes	Recurrent URTI, pneumonia	ITP, Hashimato thyroiditis	Splenomegaly, histiocytosis	Steroids, cyclosporin a, abatacept	Chemotherapy, IVIG
P15	44/F	4	44	Yes	Recurrent URTI, pneumonia	Hypogonadotropic hypogonadism, AHA, ITP, osteoporosis, fractures, periportal cavernomatous lesions, chronic atrophic gastritis, enteropathy	Splenomegaly	Steroids, cyclosporin a, abatacept	Splenectomy, IVIG

AIHA autoimmune hemolytic anemia, *ASA* acetyl salicylic acid, *ITP* immune thrombocytopenia, *EBV* Epstein–Barr virus, *GI* gastroenteritis, *DNT* double negative t cell, *FTT* failure to thrive, *IVIG* intravenous immunoglobulin, *MMF* mycophenolate mofetil, *ND* not done

Table 2 Some of the immunological tests of patients with "probable ALPS" at initial and last visit

Patient	DNT (%)	> 6-month lymphoproliferation	First ALS	First IgG	First CD19	Last IgG	Last CD19
1	2	Yes	2600	1250	13	571	13
2	3	Yes	2000	1360	11	905	14
4	10	Yes	6944	1180	25	453	5
5	10	Yes	12,500	1010	14	712	1
6	2	Yes	1400	1090	13	1110	8
9	3	Yes	1800	1150	15	1510	4
14	5	Yes	1900	1200	10	399	4

DNT double negative T cells ($TCR^+ CD4^- CD8^-$), *ALS* absolute lymphocyte count

BK virus (10^6 copy/ml) was isolated from urine and blood by PCR during the analysis of nephropathy in P8. However, it could not be documented in renal biopsy specimen. Cidofovir therapy was planned, but the urine BK viral load gradually declined during intravenous immunoglobulin (IVIG) therapy. Reactivation of BK virus while under immunosuppressives was recorded in P8 after HSCT.

Lymphoproliferative and Malignant Diseases Splenomegaly was seen in nearly all patients (14/15, 93.3%). Splenectomy was performed for 3 of them, because of hypersplenism. Lymphoma was the most common malignancy recorded in 4/15 (26.6%) patients (P5, 8, 10, 13); in three patients, non-Hodgkin (follicular) lymphoma (1), B cell lymphoma (2), and in one Hodgkin lymphoma (lymphocyte-rich) were noted. Gastric adenocarcinoma occurred in P9. Histiocytosis was documented in P14. All have taken steroids intermittantly before they got the diagnosis of malignancy. Rituximab was included in the chemotherapy regimens of some of the patients.

Gastrointestinal Manifestations Chronic diarrhea was a common symptom. It was recorded in 8/15 (53.3%) of the patients. Five (P2, 4, 8, 9, 12, 15) were diagnosed with inflammatory bowel disease. Endoscopic colon biopsy from P12 revealed CMV colitis. Two patients were diagnosed with celiac disease (P8, 9).

In three patients (P2, 4, 12), the diarrhea was so severe that it caused electrolyte imbalance (hypocalcemia, hypomagnesemia, hypokalemia) and hypovolemic shock. Two relatives of P12 who had IBD-like symptoms were also evaluated and lost at a different center. The cousin of P14 was also evaluated for IBD in another center. P9 had intramucosal adenocarcinoma developed in the basis of tubulovillous adenoma.

Autoimmune Diseases Autoimmune cytopenia (autoimmune hemolytic anemia (AHA) and immune thrombocytopenic purpura (ITP)) was frequent (6/15 and 11/15 respectively). Evans syndrome was present in 26.6% (4/15) patients. Autoimmune thyroiditis was seen in 20% (3/15) of the patients. Anti-thyroid peroxidase, anti-thyroglobulin, and anti-TSH antibodies were in high levels in P2, P4, and P14.

Immunophenotypic Analysis Results Lymphopenia was present in 30% (5/15) of the patients. A total of 4/15 (26.6%) had intermittant neutropenia and eosinophilia each (Supplementary Table 2).

66.6% (10/15) of the patients had low IgA, 40% (6/15) had low IgG, and 26.6% (4/15) had low IgM. IgG Levels progressively decreased in the patients initially diagnosed with probable ALPS

According to flow cytometric analysis, T cell numbers were low in 53.3% (8/15), and $CD4^+$ T cell numbers were low in 40% (6/15) of the patients. B cell percentage and numbers were low in 9 out of 15 (60%) of the patients (Supplementary Table 2). B cell percentage and numbers progressively decreased in the patients initially diagnosed with probable ALPS.

Double-negative T cells ($TCR\alpha\beta^+CD4^-CD8^-$) were measured in 11 out of 15 patients. Eight out of 11 (72.7%) were elevated (> 1.5%) [10].

T and B cell subgroups were evaluated in 6 and 4 patients respectively (Supplementary Tables 3 and 4). Decreased naive and recent thymic emigrant (RTE) Th cells and markedly increased effector memory and effector memory RA^+ Th cells (TEMRA Th) were documented. Normal naive B cells, but decreased switched memory B cells, were documented.

LRBA expression was low in P8 and P14 (Fig. 2a and c). P8 had low CTLA-4 and CD25 expression on $Foxp3^+$ cells, compared to controls (Fig. 2a). P8 had a large PD-1^+ population, predominant memory T cells ($CD4^+CD45RA^-$), and significantly more Tfh cells ($CD4^+CXCR5^+PD1^+$) (Fig. 2b). P14 had low CTLA-4 expression (Fig. 2c).

Western Blot Analysis Results Western blot (WB) analysis was performed for patients with CVID. The analysis was performed also for patients who were found to have LRBA mutations by WES or PID panel NGS techniques. In total, eight patients (P4, 6, 8, 9, 10, 12, 13, 14) were evaluated with WB analysis; all but P13 had no LRBA protein expression. This

Table 3 The analysis of the mutations in our cohort (The defects identified and/or published previously are shown in the 8th column)

Patient	Defect (cDNA)	Defect (protein)	Affected exon	Affected domain	The test used for diagnosis	Functional tests to document LATAIE	Defect	MAF
P1	c.5805delT	C1935Wfs*4	Exon 34	DUF1088	WES	–	Novel	< 0.01
	c.7042C>T	R2348*	Exon 45	BEACH			Identified previously [17]	< 0.01
P2, 3	c.675G>A	W225*	Exon 3	ConA and LamG	WES	Low LRBA protein expression	Published previously[15]	< 0.01
P4	c.5527delT	C1843Afs*2	Exon 31	Between A type and DUF1088	PID panel	No LRBA on WB	Novel	< 0.01
P5	c.3396_3397delAC	N1132Lfs*8	Exon 23	Between A type and DUF1088	WES	–	Novel	< 0.01
P6, 7	c.7042C>T	R2348*	Exon 45	BEACH	WES	No LRBA on WB	Identified previously[17]	< 0.01
P8	c.7041G>T	W2347C	Exon 45	BEACH	PID panel	No LRBA on WB	Novel	< 0.01
P9	IVS6+1delT		Between exons 6–7	–	WES	No LRBA on WB, low CTLA-4 surface expression	Novel	< 0.01
P10	c.3811C>T	R1271*	Exon 23	Between A type and DUF1088	PID panel	No LRBA on WB, low CTLA-4 surface expression	Novel	< 0.01
P11	c.5504delT	L1835fs*1	Exon 31	Between A type and DUF1088	PID panel	–	Novel	< 0.01
P12	c.2893_2900delinsGCCAGATATATATATATATATATATA	I964Afs*32	Exon 23	DUF1088	PID panel	No LRBA on WB	Novel	< 0.01
P13	c.175G>T	E59*	Exon 2	Before ConA	PID panel	–	Identified previously [18]	< 0.01
P14	c. (501+1_502-1)_(733+1_734-1)del	G75_W183*	Exon 3 and exon 4	Before ConA	PID panel	Low CTLA-4 surface expression, low LRBA expression	Novel	< 0.01
P15	c.2836_2839delGAAA	G946*	Exon 23	DUF1088	PID panel	Low LRBA expression	Novel	< 0.001

*The novelty for the mutations were evaluated by using gnomAD and ENSEMBLE databases

Table 4 HSCT characteristics of the patients

Patient	Age at HSCT (year)	Time from genetic diagnosis to HSCT (months)	Donor	Stem cell source	Conditioning regimen	GVHD prophylaxis	Acute GVHD	Chronic GVHD	Other complications	Follow-up after HSCT (year)	Outcome
2 [15]	7	3	MFD	BM	Bu, flu, ATG	NA	No	No	CMV, ITP, AHA	2	Alive
4 [16]	13	3	MFD	BM	Bu, flu, ATG	Mtx, cyc a	No	No	Thrombocytopenia	4	Alive
6 [16]	19	4	MFD	BM	Bu, flu, ATG	Mtx, cyc a	No	No	–	3	Alive
8	28	4	MFD	BM	Bu, flu, ATG	Mtx, cyc a	No	Yes	Recurrent pneumonia	2	Alive
9	27	5	MFD	BM	Bu, flu, ATG	Mtx, cyc a	No	No	–	1,5	Alive
10	12	12	MFD	BM	Bu, flu, ATG	Mtx, cyc a	No	–	–	0.3	Alive
12	16 [16]	4	MFD	BM	Mel, flu, ATG	Mtx, cyc a	No	No	–	3	Alive

MFD matched family donor, *BM* bone marrow, *Bu* busulphan, *flu* fludarabin, *ATG* anti-thymocyte globulin, *Mtx* metothrexate, *cyc a* cyclosporin a, *NA* not applicable

Patient 2 []

Patient 4, 6, 12 [31]

result would need to be repeated in case of sample error. However, the test could not be repeated as P13 has died.

Some of the donors were also assessed for LRBA protein expression by WB. They were found to express LRBA. In addition, post-transplant LRBA protein expression was shown by WB in two patients (P4 and P12). Figure 2d shows the result for P8 and P14. Figure 2e shows LRBA protein expression after HSCT.

Gene Defects in Patients Seven (P1, 2, 3, 5, 6, 7, 9), eight (P4, 8, 10, 11, 13, 14, 15), and one patients (P12) were diagnosed with the help of WES, PID gene panel screening, and Sanger sequencing respectively (Table 3) (Fig. 3). The defects diagnosed by WES and PID gene panel screening were confirmed by Sanger sequencing.

One patient (P1) had compound heterozygous mutations, and the others had homozygous defects. P3 was asymptomatic although her brother (P2) with the same homozygous defect was severely affected. All of the defects are confirmed to be inherited from the parents. All the defects found by various analyses were consistent with the clinical phenotype of the patients.

Genotype–Phenotype Correlation Genotype and phenotype correlation was not clear in our cohort. P4 and P12 who had severe gastrointestinal features had frameshift defects affecting the DUF1088 domain. However, P2 who had an early N-terminal mutation also had severe inflammatory bowel disease-like symptoms. The sister of P2 and P3 did not have any symptoms despite having the same defect. P13 and 14 had mutations leading to a stop codon before the ConA domain. P13 had CVID-like features, whereas P14 had ALPS-like features. P1, P6, P7, and P8, who had defects affecting the BEACH domain, had presented with cytopenia and splenomegaly.

Therapeutic Approach All the patients were given monthly IVIG and prophylactic antibacterial therapy after molecular diagnosis.

Immunomodulatory Therapies Steroids, cyclosporin A, mycophenolate mofetile, and IVIG (2 g/kg) were commonly used therapies given during severe, recurrent and relapsing autoimmune cytopenia, and inflammatory bowel disease. Eleven patients (P1, 2, 4, 5, 6, 8, 9, 11, 12, 14, 15) were given anti-CTLA-4 antibody (abatacept) therapy. P12 experienced a severe pneumonia with pleural effusion and respiratory distress after the therapy was started; so, the therapy was stopped. The others benefited from the therapy. None developed malignancy during or after abatacept therapy.

Hematopoietic Stem Cell Transplantation Seven (P2, 4, 6, 8, 9, 10, 12) out of fifteen patients (46.6%) (4/7 (57.1%) of ALPS-LRBA and 3/8 (37.5%) of CVID-LRBA patients) were

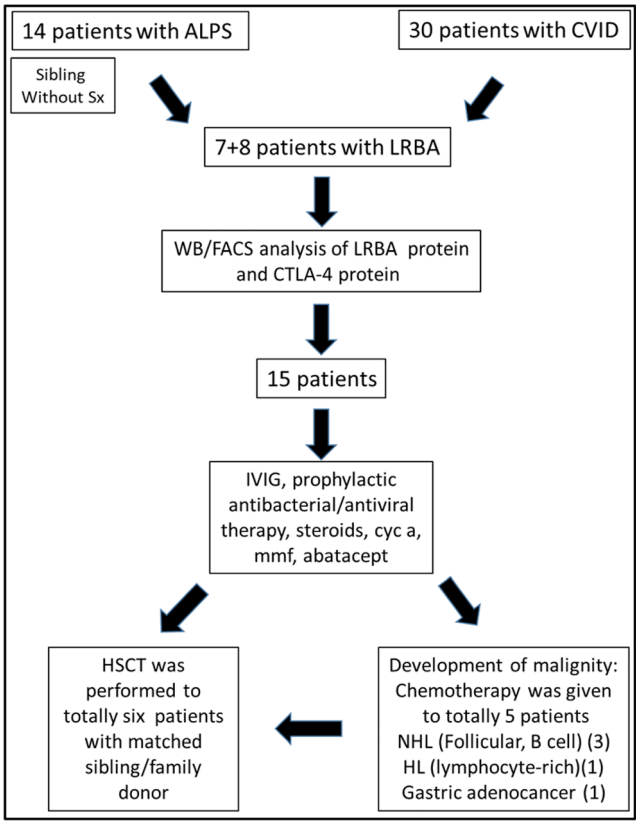

Fig. 1 Some of the patients initially followed with the diagnosis of ALPS and CVID were found to have LRBA defect

treated with hematopoietic stem cell transplantation (HSCT) from their HLA-compatible family donors (Table 4). HSCT was performed to six of them in Hacettepe University. Five (P2, 4, 6, 10, 14) were children, and two (P8, 9) were adults; P2 was transplanted in a different center [].

P4, 6, and 10 were given busulfan 0.8 mg/kg (2 days intravenous (iv)), fludarabine (160 mg/m^2 6 days and iv.), and ATG-Fresenius infusion (30 mg/kg for 2 days iv). In addition, methotrexate (10 mg/m^2 day 1 and 5 mg/m^2 on days 3, 6, 11 iv) and cyclosporine 3 mg/kg (iv) were used as GvHD prophylaxis. As P14 had severe chronic pulmonary symptoms, he was given melphalan (140 mg/m^2 (2 days intravenous (iv)), fludarabine (50 mg/m^2 6 days and iv.), and ATG-Fresenius infusion (5 mg/kg for 2 days iv) as reduced intensity regimen.

Among two adult patients, P8 and P9 were given busulfan 0.8 mg/kg 2 days, fludarabine 50 mg/m^2 6 days, and IV ATG-Fresenius infusion 5 mg/kg for 2 days. In addition, IV methotrexate 10 mg/m^2 day 1 and 5 mg/m^2 on days 3, 6, 11 and cyclosporine 3 mg/kg IV infusion were used as GvHD prophylaxis. Trimethoprim/sulfamethoxazole (po), valacyclovir (po, 1000 mg, 3 times daily), fluconazole (400 mg/day), and levofloxacine (500 mg/day) were used for antibiotic prophylaxis. Patients 8 and 9 tolerated the conditioning regimen well with no major complications. Post-transplant neutrophil and thrombocyte engraftment were at days 11 and 12–13 respectively in P8 and P9 respectively. Near full donor chimerism was achieved in post-transplant period in P8 (97%) and P9 (94%) respectively.

Four out of seven patients (P2, P4, P8, P9) were given abatacept therapy during HSCT therapy and 3–6 months after HSCT. P4 had persistant thrombocytopenia after HSCT for about 6 months and needed eltrombopag therapy. He is now well without any complication of autoimmune cytopenia.

Outcome

Seven patients that were transplanted are alive and well after HSCT (median 2 years (1-3). P4 had persistant thrombocytopenia during the first 6 months of HSCT and P8 had pulmonary GVHD, needed immunossuppressive therapy, and had intermittant BK virus reactivation before and after HSCT, which responded IVIG therapy. P2 and P6 had acute GVHD which resolved in the first year. P9 and P12 are well without major complication. P10 will be transplanted from his HLA full-matched father after the lymphoma chemotherapy. Others are well under immunomodulatory therapies.

Discussion

LRBA molecule regulates CTLA-4 expression [5], which is a potent inhibitory molecule expressed by activated T cells and regulatory T cells. It blocks co-stimulation/proliferation of the T cells and regulates immune responses [20]. Low expression of CTLA-4, FoxP3, and CD25 in LRBA deficiency results in partial loss of the regulatory effects on T cell activation, leading to increased, but inappropriate activation of T and B cells and defect in immune surveillance leading to increased cancer risk and autoimmunity [11, 21].

In this study, it was interesting to identify out patients with LRBA defects both in ALPS and CVID patient cohorts. So, the criteria of ALPS should be revised to exclude the genetic defects, such as LRBA deficiency and CTLA-4 deficiency. Elevated DNT cells in LRBA defect [22] (8/10 patients in our study) and autoimmune/inflammatory diseases, such as systemic lupus erythematosus or juvenile idiopathic arthritis [23], are considerable.

Splenomegaly and malignancy ratio was also high in this cohort. Malignancy risk is known to be increased in PIDD, and its incidence is the second-highest cause of death after infection [21]. The vast majority of lymphomas reported in PIDD are B cell lymphomas [21]. In this cohort, the ratio was 26.6% and non-Hodgkin lymphoma, Hodgkin lymphoma, and gastric adenocarcinoma were noted in patients respectively. We do not know the relation of the steroid use and malignancy. However, most of the patients used steroids for recurrent autoimmune cytopenia and IBD-like disease before the development of malignancy. There is no patient identified

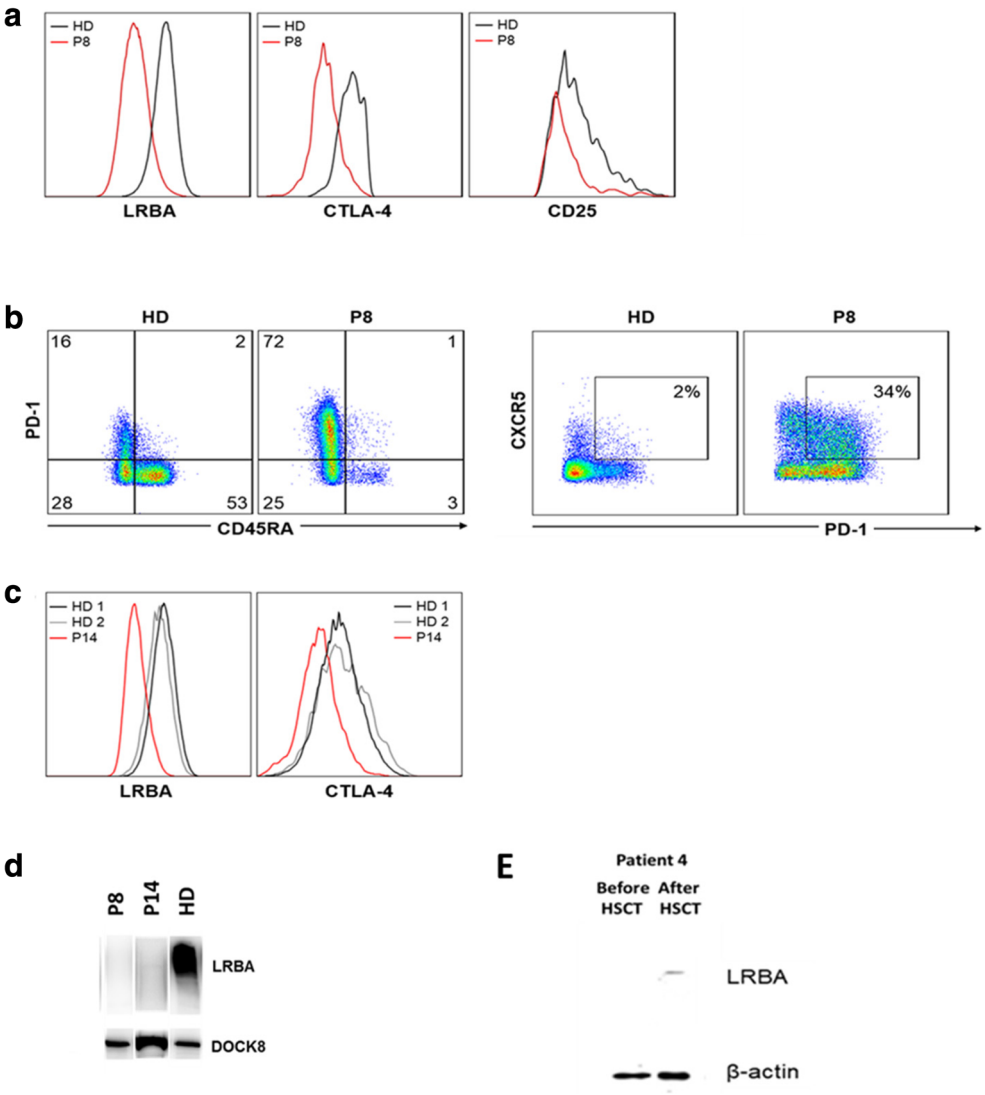

Fig. 2 Functional tests for LRBA defects. **a** Low LRBA, CTLA-4 and CD25 expression of $CD4^{+}FoxP3^{+}$ T cells in P8 compared to healthy donor (HD). **b** More memory ($CD45RA^{-}$) $CD4^{+}$ T cells are present, and T follicular helper ($CD4^{+}CXCR5^{+}PD\text{-}1^{+}$) cells are increased in P8 ($CD4^{+}$ gated cells are shown), compared to HD. **c** Low LRBA and CTLA-4 expression of $CD4^{+}FoxP3^{+}$ T cells in P14, compared to HD1 and HD2. **d** Western Blotting: No LRBA protein is seen in P8 and P14 compared to healthy donor. **e** Western Blotting: LRBA protein in P4 before and after HSCT

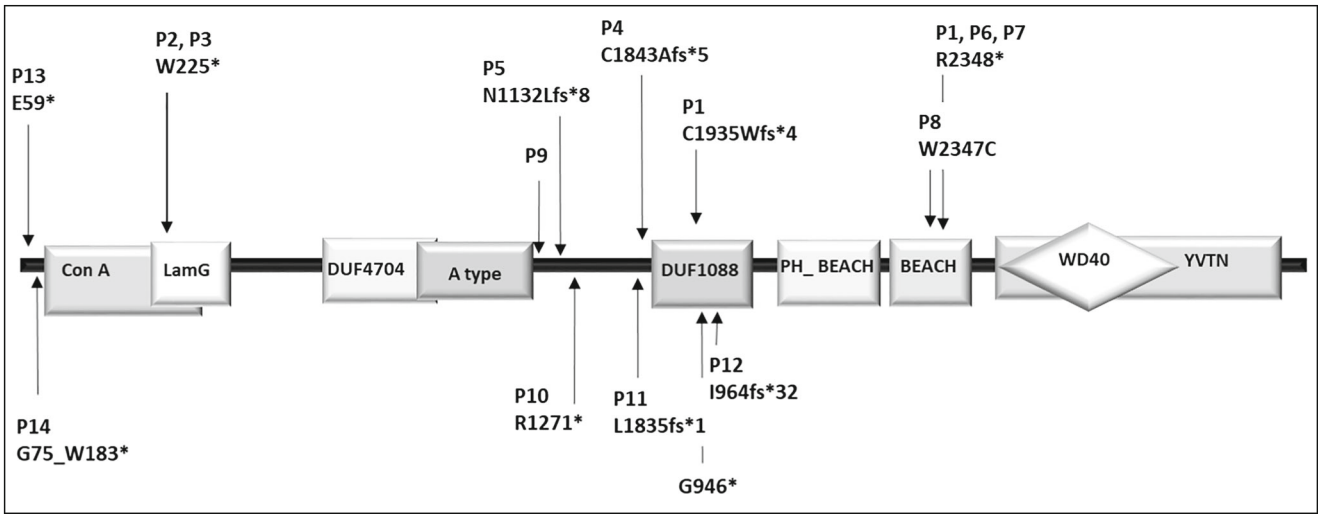

Fig. 3 LRBA protein map that illustrates its possible multidomain sites. ConA conconavalin A-like lectin/gluconase domain, DUF domain of unknown functions 4704, A type Armadillo type fold, PH PH domain associated with Beige/BEACH, BEACH Beige and Chediak Higashi, YVTN WD40/YVTN repeat–like containing domain (The figure is drawn by the help of Ensemble, PFAM, Gen3D, superfamily, SMART databases.)

among these patients having malignancy that may be related with or secondary to abatacept therapy.

Totally, 78.6% of the patients presented with autoimmune cytopenia, and 26.6% had Evans syndrome. Two (14.3%) had autoimmune thyroiditis. Other autoimmune/inflammatory features associated with LRBA deficiency are juvenile idiopathic arthritis [22], type I diabetes, erosive polyarthritis, autoimmune thyroiditis, AHA, and ITP [24]. Johnson et al. identified 4 patients with LRBA mutations having neonatal diabetes [] as in IPEX syndrome.

Autoimmune enteropathy, celiac disease, and IBD-like disease are common in the course of LRBA deficiency. Gastrointestinal pathologies may include subtotal or partial villous atrophy, or be completely normal [22]. IVIG replacement is generally insufficient to control the diarrhea. So far, corticosteroids, empirical antibiotic treatments, gluten-free diet, and various immunosuppressive agents (azathiopurin 6-mercaptopurine, tacrolimus, infliximab, rituximab, etc.) have been tried [6]. In recent publications, sirolimus was reported to be successfully used for enteropathy in patients with LRBA deficiency and to provide clinical remission and weight gain in the patients [25]. In our cohort, half of the patients presented with IBD-like symptoms, and steroids, acetyl salicylic acid, and cyclosporin A were given to control the severe symptoms.

Interestingly, endoscopic biopsy from P12 revealed CMV colitis. BK virus was isolated from urine during nephropathy in P8. BK virus–related urinary problems are usually seen in transplanted, HIV positive patients, and patients under immunosuppressive therapy [26]. Thus, the patients with chronic diarrhea, severe resistant cytopenia, and severe viral infections should be transplanted urgently, if they have matched donor. Respiratory complications, such as atelectasia, bronchiectasis, and pulmonary nodules, are commonly seen in patients with LRBA deficiency [9]. Thus, reduced intensity conditioning regimens are preferred during HSCT procedure.

Investigation at both the genetic and protein levels should be the aim for proper evaluation and diagnosis. Analysis of protein either by WB or by flow cytometry is required especially in cases where missense LRBA variants of unknown significance are detected. Nearly all reported LRBA patients thus far have impaired LRBA expression [22]. There are sometimes technical problems during WB analysis, since the large LRBA protein appears to easily degradate during sample lysate preparation. Repeating tests with positive/negative control samples may help during the analysis. Patient 13 was found to have normal protein. It was seen later that the P13's mutation was reported to associate with loss of LRBA in WB study [27]. We thought that this may be due to the binding site of the monoclonal antibody. However, measurement of CTLA-4 levels could not be done as she died.

LRBA-deficient B cells show defective activation/survival [2]. Flow cytometry reveals reduced B cell numbers [4], and reduction of IgG^+ and IgA^+ $CD27^+$ switched memory B cells in symptomatic patients. Patients have low $CD4^+CD45RA^+$naive T cells, while the percentage of naive CD8 T cells is normal. Treg cell deficiency in LRBA is associated with dysregulated T cell activation and skewing toward a memory phenotype. T cell subgroups shows decreased recent thymic emigrants but increased TEMRA cells [11]. In the present study, progressive decrease in B cells and IgG levels was seen during follow-up of patients, especially those initially diagnosed as probable ALPS. B cell subgroups showed normal naive B cells but decreased switched memory B cells. T cell subgroups showed decreased naive Th cells, RTE Th cells, markedly increased $CD4^+$ effector memory cells, and increased TEMRA Th cells. P8 and P14 showed low CTLA-4 and CD25 expression on $Foxp3^+$ cells compared with control. In P8, a large $PD\text{-}1^+$ population, increased memory, and enlarged Tfh population in the $CD4^+$ T cell compartment were recorded.

All but one patient had homozygous defects consistent with high rate of consanguinity. Compound heterozygous defect in one may be indicative of high frequency of LRBA gene heterozygosity in our country. We documented ten novel defects (Table 3). Among several domains (Fig. 3), BEACH and DUF1088 domains are the most frequently affected domains in this cohort. These are shared with another 'Beige and Chediak Higashi' (BEACH) domain containing protein (BDCP) and neurobeachins (NBEA). BDCPs are generally related to lysosome size, apoptosis, autophagy, granule size, or synapse formation []. NBEA are known to be localized near Golgi apparatus and have important roles in vesicular trafficking, intracellular transport, membrane dynamics, endosomal recycling, and receptor signaling [28]. Common two domains seem to indicate common structure/function of the LRBA and NBEA (Fig. 4).

Although the brother with the same homozygous defect was severely affected, P3 was asymptomatic. The incomplete penetrance is not reported for LATAIE disease, unlike for CTLA-4 haploinsufficiency [29]. There may be modifying genes, or exposomal effects, possibly in the healthy sister, or the symptoms will appear in the following years.

Before the molecular diagnosis, steroids, cyclosporin A, and mycophenolate mofetile were used with partial success in patients with autoimmune/lymphoproliferative manifestations. Abatacept was helpful in the patients; however, one patient had severe infectious episode (with severe respiratory distress) at the beginning of the therapy. The follow-up of the patients with LRBA deficiency is challenging even after abatacept and HSCT. Azizi et al. reported that sirolimus led to the improvement in the control of the diarrhea in four patients with LRBA deficiency [25]. Some of the patients were reported to have autoimmune clinical features also after HSCT [30].

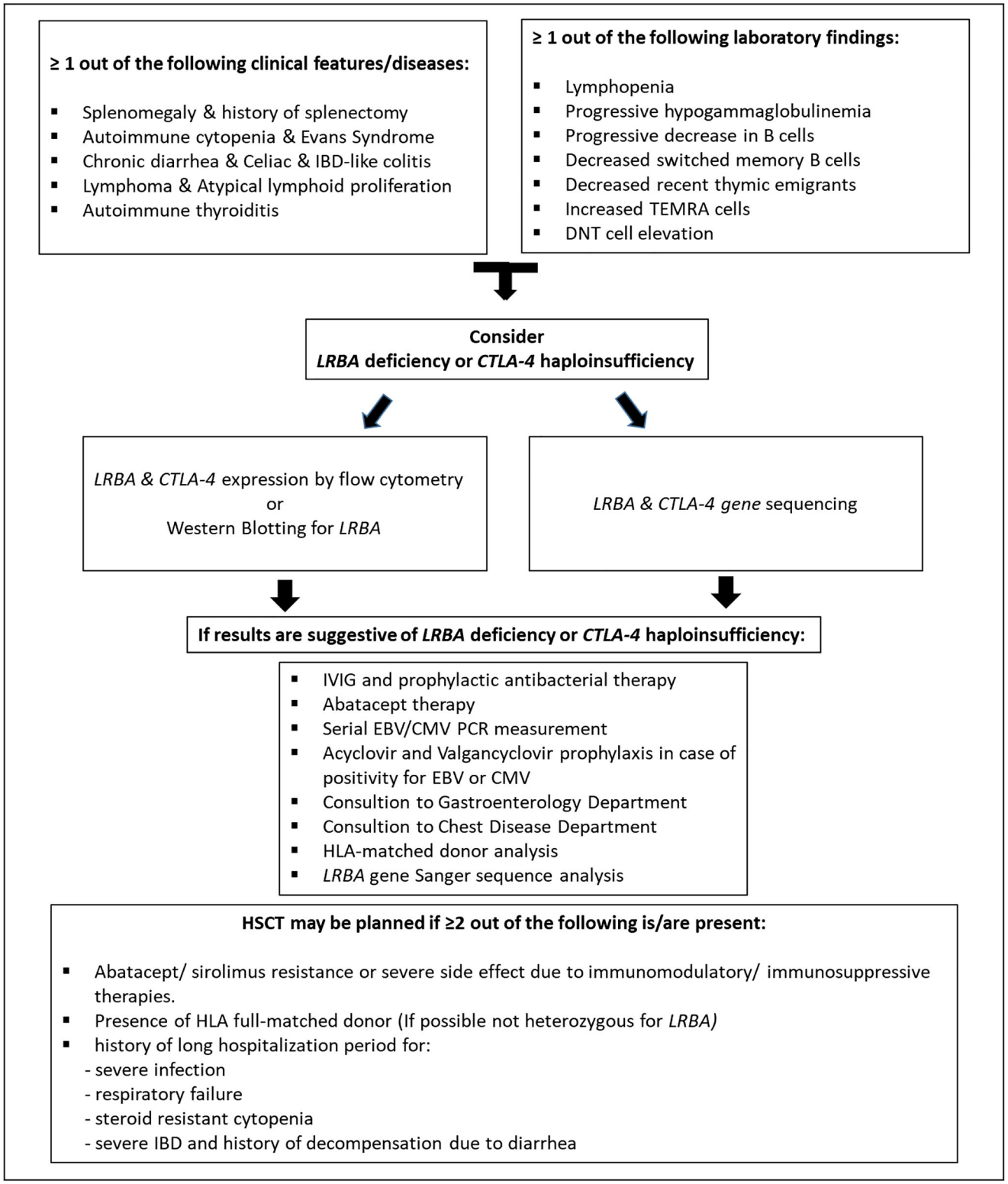

Fig. 4 Algorithm for the diagnosis and follow-up of patients with LRBA defect

In P4, autoimmune thrombocytopenia continued after HSCT. In addition to abatacept, he needed eltrombopag during an episode of thrombocytopenia.

Analysis of protein either by WB or by flow cytometry has prime importance in the diagnosis. The patients with LRBA deficiency having normal LRBA expression possibly have

low CTLA-4 levels. So, it is important to do surface staining for CTLA-4.

In this study, we have evaluated the LATAIE patients who were diagnosed among two different cohorts of CVID and ALPS patients and prepared an algorithm to ease the diagnosis and follow-up of the patients with LRBA deficiency (Table 4). In the ALPS cohort, it was very interesting to see that nearly half of the patients were found to be LRBA defective. CD19 and immunoglobulins progressively decreased during the follow-up of most of the patients with the initial diagnosis of probable ALPS. Since LRBA deficiency has only been defined relatively recently and because of the diversity of the clinical features, there is a diagnostic delay in some of the patients similar to other PID cases [31]. Next-generation sequencing aids in the diagnosis.

Acknowledgments We are thankful to our patients and families. We appreciate our laboratory staff Ozlem Karapınar, Mehtap Sonmez, Menekse Kocak and our nurses Meliha Erol and Feride Ozkan. This work was supported by the Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health. We thank Merck and Co. for support.

Funding Information This study includes the data of three studies (Project codes: 013 D08103 001-341, 11/19-23, and 16/9087) which are funded by Hacettepe University Coordination Unit for Scientific Research Projects. The study is additionally funded by TUBITAK.

Compliance with Ethical Standards

The studies were approved by the Institutional Review Board of Hacettepe University.

Conflict of Interest The authors declare that they have no conflict of interest

References

1. Alangari A, et al. LPS-responsive beige-like anchor (LRBA) gene mutation in a family with inflammatory bowel disease and combined immunodeficiency. J Allergy Clin Immunol. 2012;130(2):481–488. e2.
2. Lopez-Herrera G, et al. Deleterious mutations in LRBA are associated with a syndrome of immune deficiency and autoimmunity. Am J Hum Genet. 2012;90(6):986–1001.
3. Bogaert DJ, et al. Genes associated with common variable immunodeficiency: one diagnosis to rule them all? J Med Genet. 2016;53(9):575–90.
4. Revel-Vilk S, et al. Autoimmune lymphoproliferative syndrome-like disease in patients with LRBA mutation. Clin Immunol. 2015;159(1):84–92.
5. Lo B, et al. CHAI and LATAIE: new genetic diseases of CTLA-4 checkpoint insufficiency. Blood. 2016;128:1037–42. https://doi.org/10.1182/blood-2016-04-712612.
6. Serwas NK, et al. Atypical manifestation of LRBA deficiency with predominant IBD-like phenotype. Inflamm Bowel Dis. 2014;21(1):40–7.
7. Burns SO, et al. LRBA gene deletion in a patient presenting with autoimmunity without hypogammaglobulinemia. J Allergy Clin Immunol. 2012;130(6):1428–32.
8. Alroqi FJ, et al. Exaggerated follicular helper T-cell responses in patients with LRBA deficiency caused by failure of CTLA4-mediated regulation. J Allergy Clin Immunol. 2018;141(3):1050–1059.e10.
9. Gámez-Díaz L, et al. The extended phenotype of LPS-responsive beige-like anchor protein (LRBA) deficiency. J Allergy Clin Immunol. 2016;137(1):223–30.
10. Oliveira JB, et al. Revised diagnostic criteria and classification for the autoimmune lymphoproliferative syndrome: report from the 2009 NIH International Workshop. Blood. 2010;116(14):e35–40. https://doi.org/10.1182/blood-2010-04-280347.
11. Lo B, et al. Patients with LRBA deficiency show CTLA4 loss and immune dysregulation responsive to abatacept therapy. Science. 2015;349(6246):436–40.
12. Ozen A, et al. CD55 deficiency, early-onset protein-losing enteropathy, and thrombosis. N Engl J Med. 2017;377(1):52–61.
13. Willmann, K.L., et al. Biallelic loss-of-function mutation in NIK causes a primary immunodeficiency with multifaceted aberrant lymphoid immunity. Nat Commun. 2014;5:5360
14. Al-Mousa H, et al. High incidence of severe combined immunodeficiency disease in Saudi Arabia detected through combined TREC and next generation sequencing of newborn dried blood spots. Front Immunol. 2018;9:782.
15. Kostel Bal S, et al. Multiple presentations of LRBA deficiency: a single-center experience. J Clin Immunol. 2017;37(8):790–800.
16. Okur F, et al. Bone marrow transplantation with favorable outcome in three patients with LPS-responsive beige-like anchor (LRBA) deficiency. Clin Immunol (Orlando, Fla). 2019;203:162.
17. Johnson MB, et al. Recessively inherited LRBA mutations cause autoimmunity presenting as neonatal diabetes. Diabetes. 2017;66(8):2316–22. https://doi.org/10.2337/db17-0040.
18. Cullinane AR, Schaffer AA, Huizing M. The BEACH is hot: a LYST of emerging roles for BEACH-domain containing proteins in human disease. Traffic. 2013;14(7):749–66.
19. Tan Ç, et al. Polymorphisms in FAS and CASP8 genes may contribute to the development of ALPS phenotype: a study in 25 patients with probable ALPS. Turk J Pediatr. 2015;57(2):141.
20. Lee K-M, et al. Molecular basis of T cell inactivation by CTLA-4. Science. 1998;282(5397):2263–6.
21. Riaz IB, et al. A systematic review on predisposition to lymphoid (B and T cell) neoplasias in patients with primary immunodeficiencies and immune dysregulatory disorders (inborn errors of immunity). Front Immunol. 2019;10:777.
22. Alkhairy OK, et al. Spectrum of phenotypes associated with mutations in LRBA. J Clin Immunol. 2016;36(1):33–45.
23. Tarbox JA, et al. Elevated double negative T cells in pediatric autoimmunity. J Clin Immunol. 2014;34(5):594–9.
24. Lévy E, et al. LRBA deficiency with autoimmunity and early onset chronic erosive polyarthritis. Clin Immunol. 2016;168:88–93.
25. Azizi G, et al. New therapeutic approach by sirolimus for enteropathy treatment in patients with LRBA deficiency. Eur Ann Allergy Clin Immunol. 2017;49(5):235–9.
26. Jung SW, et al. BK virus-associated nephropathy with hydronephrosis in a patient with AIDS: a case report and literature review. Clin Nephrol. 2016;85(3):173–8.
27. Azizi G, et al. Polyautoimmunity in patients with LPS-responsive beige-like anchor (LRBA) deficiency. Immunol Investig. 2018;47(5):457–67.
28. Jogl G, et al. Crystal structure of the BEACH domain reveals an unusual fold and extensive association with a novel PH domain. EMBO J. 2002;21(18):4785–95.

29. Schubert D, et al. Autosomal dominant immune dysregulation syndrome in humans with CTLA4 mutations. Nat Med. 2014;20(12):1410.
30. Seidel MG, et al. Long-term remission after allogeneic hematopoietic stem cell transplantation in LPS-responsive beige-like anchor (LRBA) deficiency. J Allergy Clin Immunol. 2015;135(5):1384–1390. e8.
31. Seymour B, Miles J, Haeney M. Primary antibody deficiency and diagnostic delay. J Clin Pathol. 2005;58(5):546–7.