

Spectrum of Phenotypes Associated with Mutations in *LRBA*

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Abstract To date, several germline mutations have been identified in the *LRBA* gene in patients suffering from a variety of clinical symptoms. These mutations abolish the expression of the LRBA protein, leading to autoimmunity, chronic diarrhea, B-cell deficiency, hypogammaglobulinemia, functional T-cell defects and aberrant autophagy. We review the clinical and laboratory features of patients with *LRBA* mutations and present five novel mutations in eight patients suffering from a multitude of clinical features.

Keywords Primary immunodeficiency disorders (PID) · lipopolysaccharide responsive beige-like anchor protein (LRBA) · common variable immunodeficiency (CVID) · autoimmune disease (AID) · chronic diarrhea (CD) · hypogammaglobulinemia (HGG) · organomegaly (OM) ·

regulatory T-cells (Treg) · cytotoxic T-lymphocyte-associated protein 4 (CTLA4) · autophagy · apoptosis

Introduction

Since being recognized six decades ago, primary immunodeficiency disorders (PID) have been the subject of intense studies to elucidate their etiologies and to develop effective treatments. The identification of a multitude of gene mutations resulting in PID has dramatically increased our understanding of both the physiology and pathophysiology of the immune system. Currently, the identification of genetic defects does not necessarily allow for a more precise categorization of immune disorders, as variable phenotypic expression might be observed in patients with a given mutated gene [1]. In this review, we present a

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summary of defects related to lipopolysaccharide-responsive beige-like anchor protein (LRBA) deficiency and report eight new cases.

LRBA Protein

LRBA is a member of the BEACH-WD40 protein family and is expressed in several tissues [2–5]. Other BEACH domain containing proteins (BDCPs) have been reported in clinical disorders (Table 1) [6]. The *LRBA* gene is located on 4q31.3, contains 57 exons and encodes a protein containing 2851 amino acid residues [7]. The LRBA protein is a cytosolic protein that is expressed in several cell types including hematopoietic, neural, gastrointestinal and endocrine cells [8]. Increased expression of *LRBA* has been observed in renal, pancreatic, colorectal, lung and central nervous system cancers [9]. The human protein sequence shows a marked homology with other species including mouse (90 %), chicken (81 %) and zebrafish (73 %) [8].

The repeated WD40 domain, located at the C-terminal of LRBA, is highly conserved and is abundant in eukaryotic organisms, participating in multiple cellular processes including cytoskeleton assembly, signal transduction, vesicular trafficking, transcriptional regulation, chromatin dynamics and apoptosis [5]. It is preceded by the Plekstrin Homology (PH)-like domain and BEACH domain which have been implicated in the maintenance of intracellular stores of CTLA4 (cytotoxic T-lymphocyte-associated protein 4) in T-cells [5, 10]. The binding partners for the LRBA WD40 domain have not yet been identified [2]. It is hypothesized that the repeated motifs of the WD domain are important for signaling and protein folding [5]. However, the definitive role of LRBA in cellular function remains to be clarified.

LRBA Deficiency

Germline defects of *LRBA* (Fig. 1a) have been linked to immunodeficiency, inflammatory bowel disease (IBD)-like enteropathy and autoimmune disease (AID), including immune thrombocytopenic purpura (ITP) and autoimmune hemolytic anemia (AIHA) [2, 11, 12], while somatic mutations have been reported in breast cancer [13]. In this review, we provide a summary of the clinical and laboratory findings in 31 patients with LRBA deficiency. They include 23 patients published to date and eight additional unpublished patients in whom we recently identified novel *LRBA* mutations.

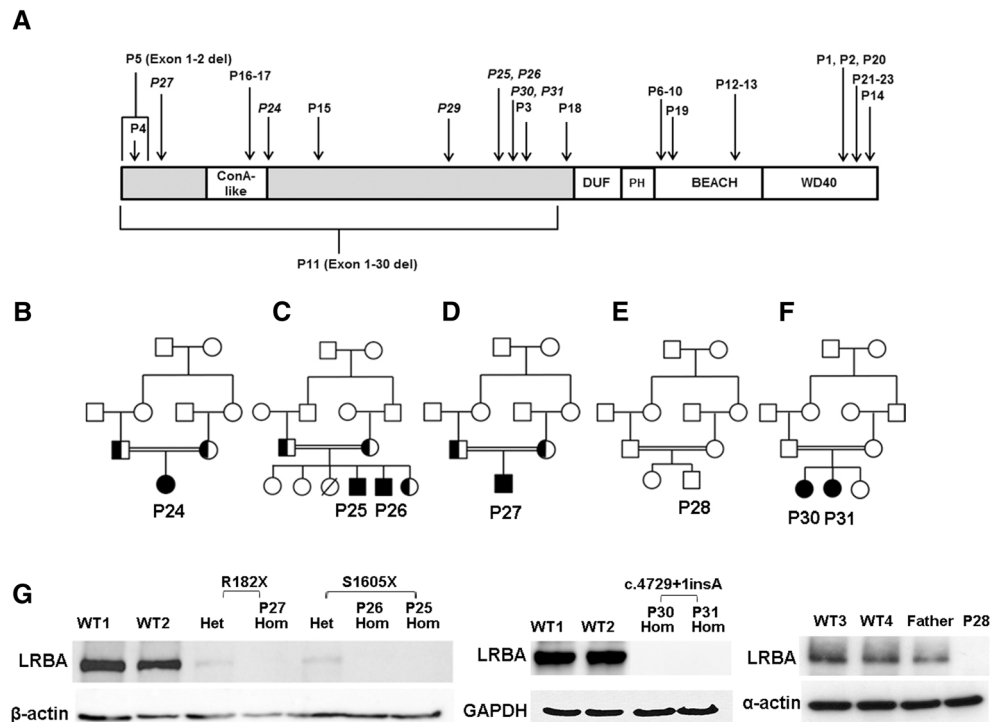
The clinical and laboratory features of patients reported with LRBA deficiency are presented in Table 2 and Supplementary Tables 1 and 2 (previously published patients (P1-P23) [2, 11, 12, 14–20] and new patients (P24-P31)). Some cases of LRBA deficiency have been reported but without comprehensive details. Thus, Dogu et al. reported on a c.C5505del T (p.I1837X) homozygous mutation in a girl (P18) born to consanguineous parents who presented with chronic diarrhea, increased frequency of respiratory infections, severe growth retardation, bronchiectasis, decreased serum IgM level, normal lymphocyte subsets and absent switched-memory B-cells [18]. Piquer et al., reported on a girl (P19) of North African descent born to consanguineous parents who presented with chronic diarrhea since early infancy and who later developed hypogammaglobulinemia [19]. Sequencing revealed a homozygous mutation c.C6607T (p.R2203X) in exon 4. Lopez-Herrera et al. reported on six additional patients not known to be born to consanguineous parents with hypogammaglobulinemia (five with non-infectious diarrhea) and absent LRBA protein expression on Western blots (WB), two of whom have homozygous mutations affecting *LRBA*, but other laboratory values were not presented [17]. Recently,

Table 1 BEACH domain containing proteins and associated disorders

Protein	Associated/reported clinical disorder	OMIM numbers
LYST	Chediak–Higashi syndrome	#214500; *606897
NBEA	De novo translocation t (5;13) (q12.1;q13.2); idiopathic autism	*604889
NBEAL1	Upregulated expression in gliomas	*609816
NBEAL2	Gray platelet syndrome	#139090; *614169
LRBA	CVID	#614700; *606453
WDFY3	None	NA
WDFY4	None	NA
NSMAF	None	*603043
WDR81	CAMRQ2	#610185; *614218

LYST lysosomal trafficking regulator, *NBEA* neurobeachin, *NBEAL* neurobeachin-like, *LRBA* lipopolysaccharide-responsive beige-like anchor protein, *WDFY* WD and FYVE zinc finger domain containing protein, *NSMAF* neutral sphingomyelinase activation-associated factor, *WDR81* WD repeat domain 81, *OMIM* online mendelian inheritance in man, *CVID* common variable immunodeficiency, *NA* not assigned, *CAMRQ2* autosomal recessive cerebellar ataxia, mental retardation, and dysequilibrium syndrome-2

Fig. 1 a. An approximate schematic representation of domains and location of mutations in LRBA at the protein level. *BEACH* domain of Beige and Chediak-Higashi, *ConA-like* concanavalin A-like lectin/glucanase domain, *DUF* domain of unknown function, *PH* Pleckstrin homology domain. *Italics* indicate novel mutations. **b-f.** Family pedigrees of new patients (*double lining*, consanguinity; *half-fill*, heterozygous; *solid black*, homozygous; *no fill*, unknown). **g.** Western blot analysis reveals the absence of LRBA expression in patients P25-28, P30 and P31, and a reduced expression in heterozygous carriers. *Hom* homozygous, *Het* heterozygous, *WT* wild type, *GAPDH* glyceraldehyde-3-phosphate dehydrogenase



Lo et al. reported 12 novel mutations in nine patients leading common variable immunodeficiency (CVID) and autoimmune disease phenotypes [10].

Of the newly identified patients, patient P24 (Fig. 1b) was the only child born to consanguinous parents of Iranian descent. She first presented at 3 years of age with upper respiratory tract infections (URTI) complicated by pneumonia necessitating hospitalization. At age 6 she developed fever, productive cough, acute otitis media, lymphadenopathy, finger clubbing, hepatosplenomegaly, wart-like skin lesions and pneumonia. Serum immunoglobulins were undetectable, and the bone marrow biopsy and aspirate were morphologically normal. A diagnosis of common variable immunodeficiency (CVID) was made and intravenous immunoglobulin (IVIG) was started. At age 7 she was diagnosed with juvenile idiopathic arthritis (JIA) and severe bronchiectasis. Computed tomography (CT) showed segmental emphysema and pulmonary function tests showed a mild restrictive and obstructive pattern with an absent response to bronchodilators. Insect bite allergy and conjunctivitis were also noted. At age 8, exacerbation of her arthritis necessitated hospitalization with occurrence of septic arthritis of the knee later the same year. Chronic diarrhea was noted at this time. She had completed her vaccination schedule without complications. She died at age 12 from respiratory failure secondary to pneumonia. Whole exome sequencing (WES) revealed a homozygous splice-site mutation c.1014+1 C>T (exon 8–9). This mutation was confirmed by Sanger sequencing in the patient, and in a heterozygous form in both her parents.

Patient P25 (Fig. 1c) is a 26-year-old man, born to consanguinous Iranian parents, who presented at 2 years of

age with recurrent sinusitis and failure to thrive. He suffered seven bouts of pneumonia from 3 to 19 years of age. Other features included infectious diarrhea (*Giardia species* and *Trichomonas hominis*), hepatosplenomegaly, lymphadenopathy, arthralgia, hyperpigmented skin lesions on the abdomen, bilateral cylindrical bronchiectasis and finger clubbing. Laboratory investigations at age 10 showed hypogammaglobulinemia (Supplementary Table 2), absent isoheamagglutinins and a normal nitro-blue tetrazolium (NBT) test. CVID was diagnosed and IVIG was started. WES revealed a novel homozygous nonsense mutation c.C4814G (p.S1605X, exon 30) which was confirmed by Sanger sequencing. A male sibling (P26) was also found to be homozygous for the same mutation, while both parents were heterozygous carriers. The homozygous brother had a history of recurrent infections, but is clinically well with recent immunological evaluation showing normal serum concentrations of immunoglobulins and specific antibody levels. WB analysis of both siblings showed absent LRBA expression (Fig. 1g).

Patient P27 (Fig. 1d) is a 14 year old boy born to first-cousins of Iranian descent. He presented at 2 years of age with URTI and required five hospitalizations for pneumonia over the following 6 years. At the age of eight he had hepatosplenomegaly, bilateral bronchiectasis, finger clubbing, and bilateral pulmonary lymphadenopathy with calcified granulomas. CVID was diagnosed at this time as serum immunoglobulin levels were reduced; and IVIG was subsequently commenced. Splenic ultrasonography revealed massive splenomegaly with calcified areas. Abdominal CT showed multiple hepatic, splenic and adrenal granulomas.

Table 2 Main clinical and laboratory features of LRBA deficiency

Patient	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	P16	P17	P18	P19	P20	P21	P22	P23	P24	P25	P26	P27	P28	P29	P30	P31					
Autoimmune disease	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+				
Hypogammaglobulinemia	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			
Respiratory infections	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Organomegaly	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Cytopenias (including TCP)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Chronic diarrhea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Other GIT features	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Other infections	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Other lung disease	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Finger clubbing	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Growth retardation	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Arthritis/arthralgia	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neurological features	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skin infection	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Atopic skin disease	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Other skin disease	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoproliferative disease	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CD4 T-cell counts	Nor	Nor	Nor	Nor	↓	Nor	Nor	Nor	Nor	Nor	Nor	Nor	Nor	Nor	Nor	↑	↑	Nor	NA	↓	↓	↓	↓	Nor	Nor	NA	Nor	Nor	Nor	Nor	Nor	Nor	Nor	Nor	Nor	
CD8 T-cells counts	Nor	Nor	↑	↑	↓	Nor	Nor	Nor	Nor	Nor	Nor	↓	Nor	Nor	Nor	Nor	Nor	Nor	NA	Nor	Nor	Nor	Nor	↑	Nor	NA	Nor	Nor	Nor	↑	Nor	Nor	Nor	Nor	↓	
B-cells counts	Nor	Nor	Nor	↓	Nor	Nor	Nor	↓	↓	Nor	↓/Nor	Nor	Nor	Nor	Nor	↓	↓	Nor	Nor	↓	↓	↓	↓	↓	Nor	NA	↓	↓	↓	↓	↓	↓	↓	↓	Nor	Nor
NK-cells counts	Nor	Nor	↓	NA	NA	Nor	Nor	Nor	Nor	Nor	↓	Nor	Nor	Nor	NA	Nor	Nor	Nor	NA	NA	NA	NA	NA	NA	Nor	NA	NA	NA	NA	NA	NA	NA	NA	NA	Nor	Nor
% Switched																																				
memory B-cells	↓	↓	↓	↓	NA	NA	NA	NA	NA	NA	↓	NA	NA	Nor	NA	NA	NA	NA	↓	↓	↓	↓	↓	NA	NA	NA	NA	↓	↓	↓	↓	↓	↓	↓	↓	NA
Treg counts	NA	NA	NA	NA	NA	NA	NA	↓	↓	↓	NA	NA	NA	NA	↓	↓	↓	NA	NA	↓	↓	↓	↓	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Treg function	NA	NA	NA	NA	NA	NA	NA	↓	↓	↓	NA	NA	NA	NA	↓	↓	↓	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

TCP thrombocytopenia, NA not available, Nor normal Treg regulatory T cell, GIT gastrointestinal tract

Portal hypertension was diagnosed at this time. One year later, although IgG levels increased with IVIG, IgG remained low and a persisting reversed CD4/CD8 T-cell ratio was present. Absent tonsils were also noted at this time. At age 12 he developed left knee joint stiffness and a decreased range of motion, at which time JIA was diagnosed and prednisolone and hydroxychloroquine were started. Serum rheumatoid factor and anti-nuclear antibodies were noted. One year later, a history of chronic diarrhea lasting for 3 years was revealed. Laboratory investigations showed *Trichostrongylus* ova and blood in the stools. Anti-parasitic therapy was started. Additionally, ulceration of the lower esophageal sphincter was found. WES revealed a novel homozygous nonsense mutation c.C544T (p.R182X, exon 4). WB showed absent LRBA expression in the patient, and decreased protein expression in his heterozygous mother (Fig. 1g).

Patient P28 (Fig. 1e) is a 10 year old Iranian boy, born to consanguineous parents. He presented at two months of age with a persistent dry cough that was unresponsive to therapy and was diagnosed with bronchial asthma four months later. From 2 to 3 years of age he was admitted to the hospital repeatedly due to symptoms related to thrombocytopenia (and later pancytopenia), requiring platelet transfusion, IVIG, granulocyte colony stimulating factor (G-CSF), cyclosporine, corticosteroids and Rituximab. Pseudomonas septicemia occurred once requiring antibiotic therapy. Other clinical features included inguinal, cervical, and abdominal lymphadenopathy, as well as hepatosplenomegaly. A bone marrow biopsy showed mild myeloid maturation arrest associated with an increase in lymphoid series. Peripheral lymphocyte analysis showed low counts of B-cells (Fig. 2a), reduced switched memory B-cells (Fig. 2b), a high percentage of transitional B-cells/plasma-blasts (Fig. 2c), and a reversed CD4:CD8 T-cell ratio (Fig. 2d). He had a normal NBT test. Cytomegalovirus (CMV) and Epstein-Barr virus (EBV) serology were positive (IgG), but the EBV viral-load was negative. At age five, CVID was diagnosed with unilateral optic nerve atrophy. Later, he required multiple hospital admissions for recurrent and persistent respiratory tract infections and suspected granulomatous-lymphocytic interstitial lung disease (GLILD); and developed chronic non-infectious diarrhea. Whole genome sequencing revealed variations in the second intron (12 non-splice site and two small insertions) but no exonic mutations in *LRBA* which were present in a region of homozygosity. WB analysis showed absence of the LRBA protein (Fig. 1g).

Patient P29 is a 6 year old Lebanese girl, born to consanguineous parents (distant relations). She presented at 3 years of age with dental abscesses, requiring surgical drainage, followed by recurrent otitis media, requiring adenoidectomy and resulting in hearing loss. At age four, she had several hospital admissions for generalized lymphadenopathy, hepatosplenomegaly and poor weight gain. A full blood count showed thrombocytopenia. Thoracic and

abdominal CT scans showed multiple bilateral pulmonary nodules and lymphadenopathy (pulmonary, retroperitoneal and mesenteric). A liver biopsy did not show any specific histopathologic pattern. Surface and abdominal lymph node biopsies did not show features of malignancy. During hospitalization, she developed seizures. Magnetic resonance imaging (MRI) of the brain revealed a hyper-intense subcortical lesion in the left parietal lobe extending to the posterior aspect of the basal ganglia and internal capsule. Cerebrospinal fluid cultures grew *Staphylococcus aureus*, for which antibiotic therapy was given. Serum IgG anti-CMV was detected. Other serological and microbial culture analyses were negative, including those for *Salmonella spp.*, *Brucella spp.*, *Toxoplasma gondii* and EBV. She received additional antibiotic therapy for a later episode of suspected pneumonia. At age five she was referred for immunological evaluation and mentioned the occurrence of intermittent arthralgias. Laboratory evaluation revealed hypogammaglobulinemia, low IgG subclass levels (IgG1, IgG2 and IgG4), normal NBT test, normal complement component levels (C3 and C4) and negative autoantibody profiles. The reduced B-cell proliferation was observed in three assays (stimulation with CpG oligonucleotides, anti-CD40/interleukin (IL)-4 and anti-CD40/IL-21). IVIG was started. She later underwent a splenectomy due to hypersplenism, and a diagnosis of Castleman's disease (hyaline-vascular type) was made based on histopathologic examination. WES identified a novel *LRBA* mutation which results in a frameshift deletion and a premature stop codon (c.2963delT; p.N988fs*7), which was confirmed by confirmed by Sanger sequencing.

Patient P30 (Fig. 1f) has been previously reported [21], but details are updated in this review. She is a 21 year old Iranian woman, born to consanguineous parents. She presented at 3 years of age with growth retardation, URTI, recurrent pneumonia, recurrent diarrhea (celiac-like disease), hepatosplenomegaly and was diagnosed with probable IgA deficiency. At age 8 she developed Hashimoto's disease. At age 13 she was diagnosed with recurrent pneumonia, bronchial asthma and atopic dermatitis. Laboratory evaluation revealed thrombocytopenia and a low serum immunoglobulin levels, indicating progression to CVID. IVIG was started. She later developed AIHA and underwent a splenectomy at age 20 due to hypersplenism. Her diarrhea failed to resolve after a gluten-free diet but resolved spontaneously after splenectomy. WES revealed a novel splice site mutation (c.4729+2insA; 3' to exon 29) which was confirmed by Sanger sequencing. Her sister (P31) is an 18 year old woman who was healthy until 5 years of age when she developed AIHA. The following year she suffered multiple URTI followed by generalized lymphadenopathy and splenomegaly. Persistence of her anemia necessitated a splenectomy at that time. At age 10, she was admitted to the intensive care unit for a severe viral meningitis. Laboratory investigation at this time showed

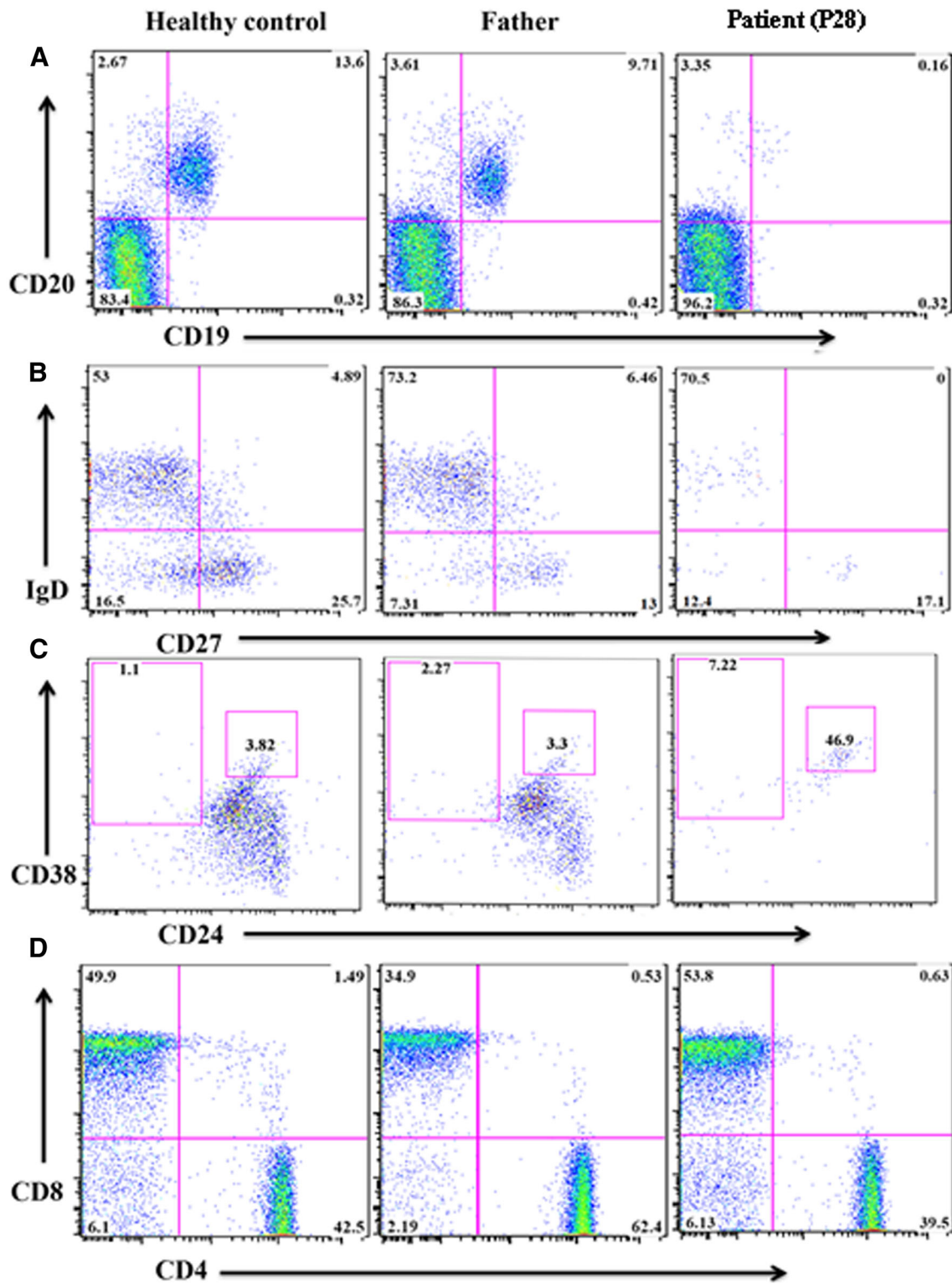


Fig. 2 Peripheral blood lymphocyte immunophenotyping of P28 compared with his father and a healthy control. **a**, B-cells; **b**, memory B-cells; **c**, B-cell subsets; **d**, T-cell subsets; and **e**, NK cells

thrombocytopenia, an elevated vitamin B12 level, increased double-negative T-cell counts, low CD8 T-cell counts and normal immunoglobulin levels. A bone marrow aspirate showed severe hypercellularity with maturational arrest in

the myeloid series. A diagnosis of autoimmune lymphoproliferative syndrome (ALPS) was made and treatment with corticosteroids was commenced. At age 11 she developed ITP and a year later she suffered another episode of viral meningitis.

Brain MRI showed a focus of abnormal signal intensity on the left side of the optic chiasm with bulging of the left optic nerve presumed to be due to a granuloma-like lesion coupled with a demyelinating process similar to that observed in multiple sclerosis, but a brain biopsy was not performed. Two years later she lost the vision on her left eye and she was started on immunomodulatory IVIG therapy. From the ages of 15 to 18, recurrent episodes of sinusitis and otitis media occurred (2–3 infections per year). WES revealed the same mutation as her sister (P30) and was confirmed by Sanger sequencing. WES also excluded genes known to be associated with ALPS, leading to designate her condition as an ALPS-like disorder.

A search for additional mutations in genes associated with immunodeficiency, autoimmune disease and lymphoproliferative disorders was conducted for previously published patients (P4, P5) and for newly reported patients (P24–P27, P30 and P31). These included mutations in genes known to be associated with a CVID-like phenotype [22] and in all other known primary immunodeficiency genes (Supplementary Table 3); mutations in genes suggested to be associated with lymphoma and lymphoproliferative diseases (Supplementary Table 4); and mutations in genes suggested to be associated with autoimmunity and autoimmune disorders (Supplementary Table 5). The sequencing depth was above 20 in the majority of these genes. The search did not identify significant mutations associated with autoimmune disease (Supplementary Table 6).

Hematopoietic stem cell therapy (HSCT) was not considered for the patients as a diagnosis of CVID was established and treatment administered accordingly. For patient P31, HSCT was not considered as her investigation is ongoing.

Clinical Phenotypes in LRBA Deficiency

The clinical features observed in patients with LRBA deficiency (Tables 2, 3, 4 and Supplementary Tables 1 and 2) are heterogeneous with age of presentation ranging from two

months to 12 years. The majority (71 %; 22/31) presented at or before 5 years of age. The disease phenotype can broadly be divided into an enteropathy phenotype, an autoimmunity phenotype and an immunodeficiency phenotype. The enteropathy phenotype includes autoimmune enteropathy, IBD/IBD-like disease and non-infectious diarrhea; the autoimmunity phenotype includes mainly AIHA and/or ITP; and the immunodeficiency phenotype includes combined immunodeficiency (CID), CVID and a CVID-like disease. There does not appear to be any genotype-phenotype correlation as patients with the same mutation may have different clinical phenotypes or even be asymptomatic (P22).

The most common features in the patients were chronic diarrhea (19/31), AID (19/31), organomegaly (19/31), respiratory tract infections (19/31) and hypogammaglobulinemia (18/31) (Tables 2 and 3). Figure 3 illustrates the proportion of patients who had one or more of the main phenotypic features, with the exception of respiratory tract features. It is apparent from Fig. 3 that a categorization of the overlap of clinical features is not feasible, as the majority of patients (84 %; 26/31) manifest two or more clinical symptoms.

No pathogen was identified in the majority of cases with chronic diarrhea (79 %; 15/19). However the protracted nature of the diarrhea suggests that even when gastrointestinal infections were present, another etiology might have contributed. Overall, growth retardation was reported in 42 % of cases (13/31). With the exception of P7 and P29, growth retardation occurred in association with chronic diarrhea. In P7, diarrhea would have significantly aggravated the effects of the growth hormone deficiency.

Autoimmunity

Autoimmune features are prominent in patients with LRBA deficiency with 19/31 (61 %) of the patients suffering from at least one autoimmune disorder. AIHA (12/31) and ITP (9/31) were the most common, followed by autoimmune thyroid disease (3/31), autoimmune enteropathy (AIE) (2/31), type 1 diabetes mellitus (2/31), and JIA (2/31). Other conditions/features occurring in individual patients included atrophic gastritis with anti-intrinsic factor antibodies (P3), myasthenia gravis (P5), vitiligo (P12), celiac disease (P18) and a celiac-like disease (P30).

Two patients developed infectious arthritis (gonococcal arthritis in P2 and P13), while rheumatologic features were present in six patients (including those with JIA). P11 developed a sterile psoas abscess, which may be related to her autoimmune enteropathy. Psoas abscesses have been reported in relation to Crohn's disease [23], but there was no mention of any fistulous communication between the abscess and intestine in the patient.

Table 3 Proportions of clinical phenotypes in LRBA deficiency

Clinical phenotype	Number of patients	Percentage of patients
Chronic diarrhea	19	61 %
Autoimmune disease	19	61 %
Organomegaly	19	61 %
Respiratory infections	19	61 %
Hypogammaglobulinemia	18	58 %
Growth retardation	13	42 %
Neurologic disease	7	23 %

Table 4 General data on patients with LRBA deficiency*

Patient*	Sex	Ethnicity	Mutation	Age of onset (years)	Main phenotype	Reference
P1	M	Arabian	p.I2657S	1	HGG, AID	[2]
P2	F	Arabian	p.I2657S	1	HGG, AID	[2]
P3	M	Sicilian	p.R1683X	12	HGG, AID, CD, OM	[2]
P4	M	Iranian	p.E59X	2	HGG, AID, CD, OM	[2]
P5	F	Iranian	Exon 1–2 deletion	2	HGG, AID, CD	[2]
P6	M	Arabian	p.E22196fs*3	0.2	CD, RTI	[12]
P7	F	Arabian	p.E22196fs*3	2	CD	[12]
P8	F	Arabian	p.E22196fs*3	4	HGG, CD, RTI	[12]
P9	F	Arabian	p.E22196fs*3	3	HGG, AID, CD	[12]
P10	F	Arabian	p.E22196fs*3	2	HGG, CD	[12]
P11	F	Pakistani	Exon 1–30 deletion	4	CD, AID	[11]
P12	F	Kurdish	p.T2388Pfs*7	2	AID, RTI	[14]
P13	F	Kurdish	p.T2388Pfs*7	5	HGG, AID, OM	[14]
P14	F	Turkish	p.I2824P	0.5	CD, AID, OM	[15]
P15	M	Arabian	p.Q678X	0.25	CD, AID	[16]
P16	M	Lebanese	p.C289Cfs*3	7	HGG, OM, RTI	[16]
P17	F	Lebanese	p.C289Cfs*3	10	OM, RTI	[16]
P18	F	Not known	p.I1837X	6	CD, AID, OM, RTI	[18]
P19	F	North African	p.R2203X	<1	HGG, CD, OM	[19]
P20	M	Palestinian	p.I2657S	3	HGG, OM, AID	[20]
P21	M	Palestinian	p.S2713fs*13	6	OM, AID	[20]
P22	M	Palestinian	p.S2713fs*13	NA	Asymptomatic	[20]
P23	M	Palestinian	p.S2713fs*13	1.5	OM, AID	[20]
P24	F	Iranian	c.1014 + 1 C > T	3	HGG, AID, OM, RTI	New patient
P25	M	Iranian	p.S1605X	2	HGG, CD, OM, RTI	New patient
P26	M	Iranian	p.S1605X	NA	RTI	New patient
P27	M	Iranian	p.R182X	2	HGG, CD, AID, OM	New patient
P28	M	Iranian	None detected	0.2	HGG, CD, OM, RTI	New patient
P29	F	Lebanese	p.N988fs*7	3	HGG, OM, RTI	New patient
P30	F	Iranian	c.4729 + 2insA	6	HGG, AID, OM, RTI	New patient
P31	F	Iranian	c.4729 + 2insA	13	AID, OM, RTI	New patient

*All patients were born to consanguineous parents

AID autoimmune disease, CD chronic diarrhea, HGG hypogammaglobulinemia, NA not applicable, OM organomegaly, RTI respiratory tract infections

Respiratory Manifestations

Respiratory tract infections occurred in 61 % (19/31) and bronchiectasis in a third of the patients (10/31). Other features included lymphoid interstitial pneumonia (LIP) (2/31), bronchial asthma (2/31) and fibrosing pneumonitis (1/31). Finger clubbing was observed in eight patients (8/31).

Lymphoproliferative Disease

Two patients developed lymphoproliferative disease. Patient P6 developed EBV related lymphoproliferative disease that

resolved spontaneously. Patient P19 developed marginal zone lymphoma related to EBV infection.

Neurologic Features

Neurological features occurred in seven patients (23 %; 7/31). With the exception of myasthenia gravis (P5), which has an autoimmune etiology, the occurrence of cerebral lesions and nervous tissue atrophy in six patients (19 %) appears alarmingly high. Two patients (P1 and P2) developed cerebral granulomas (radiologically) with serious complications (strabismus, hemiplegia and seizures), another (P31) developed a granuloma-like lesion (radiologically) with demyelination

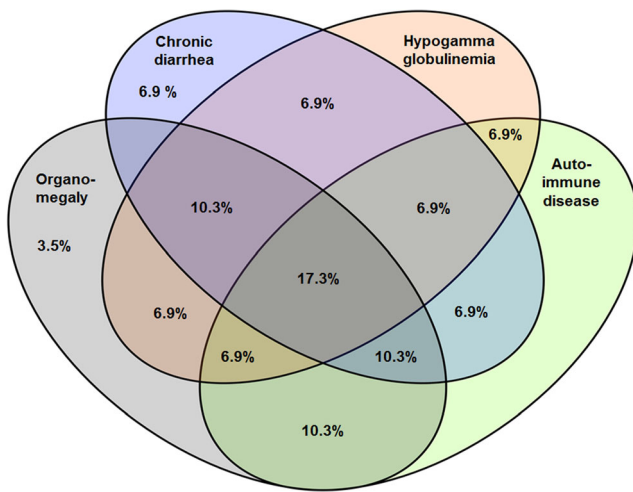


Fig. 3 Venn diagram showing the proportion of patients with multiple phenotypes, excluding P22 (asymptomatic) and P26 (URTI only)

resulting in unilateral optic nerve atrophy, a fourth (P28) also suffered unilateral optic nerve atrophy. Patient P3 suffered cerebral and cerebellar atrophy and one (P29) had a parietal lobe lesion complicated by seizures. It is interesting to observe that all patients suffering these complication were unrelated and had different mutations (Supplementary Table 2).

Other Phenotypic Features

Two siblings (P16 and P17) and the unrelated P24 had thrombocytopenia, which may suggest a subtle unidentified autoimmune component. Dermatological features included allergic features (allergic dermatitis and urticaria); infectious conditions (warts and cellulitis) and others (erythema nodosum, hyperpigmented and necrotic lesions).

Laboratory Features

Lymphocyte subsets and immunoglobulin levels are presented in supplementary table 2. T-cell counts were generally normal. Although B-cell counts were only reduced in 42 % (13/31) of the patients (including the asymptomatic P22), 58 % (18/31) had hypogammaglobulinemia. In addition, decreased IgM levels alone occurred in three (3/31) patients and low IgA levels (17/31) occurred in conjunction with hypogammaglobulinemia. Class-switched memory B-cells were absent or reduced in the all of those tested (13/14) with the exception of one patient (P14) who had a normal level. T-cell apoptosis was increased in three patients (P20, P21 and P23) with an ALPS-like phenotype [20].

Microbiologic and Histopathologic Features

There does not appear to be specific susceptibility to any particular bacterial or viral pathogen. The organisms detected

were *Staphylococcus aureus*, *Streptococcus viridians*, *Streptococcus pneumonia*, *Giardia* spp., *Trichomonas homini*, *Trichostrongylus* spp, *Haemophilus influenzae*, *Aspergillus* spp, CMV and EBV [2, 11, 20]. Regarding gastrointestinal pathology, histological features present were subtotal villous atrophy (P5 and P7), partial (duodenal) villous blunting (P6), duodenal villous atrophy (P9, P14) and normal villi (P19).

Genetics of LRBA Deficiency

The positions of known *LRBA* mutation are presented in Fig. 1a. The p.I2657S (or p.I2646S from the complete transcript) substitution (P1, P2, P20), resulted in absent expression of LRBA on WB, suggesting perturbed stability of the protein [2, 20]. The p.R1683X (P3) change, if translated, would result in a truncated protein affecting the expression of the PH, BEACH and WD40 domains; however, no LRBA expression at all was detected by WB, suggesting nonsense-mediated decay of the shortened mRNA. The p.E59X change (P4) if translated, would probably produce a short non-functioning protein [2]. The large deletions in patients P5 and P11, would eliminate the translation start site resulting in lack of expression, which was confirmed by mRNA analysis and WB for P11 [2, 11]. The p.E2219Dfs*3 substitution (P6-10), resulted in absent LRBA expression on WB [12]. The homozygous splice-site c.1014+1 C>T substitution, at the 3' end of exon 8, would probably result in exon deletion resulting in a truncated protein devoid of the BEACH and WD40 domains (WB was not possible as the diagnosis was established post-mortem). In P11, exons 1 to 30 were deleted with loss of LRBA expression on WB [11]. The c.7162delA (p.T2388Pfs*7) resulted in loss of protein expression (P13) and reduced LRBA expression in P12, who underwent hematopoietic stem cell transplantation (HSCT), receiving stem cells from her heterozygous mother [14]. For P19 (p.R2203X), WB was not performed. The p.I2824P (P14) mutation was the only case where LRBA protein was detected at a similar level as healthy controls. The p.S2713fs13 mutation in siblings P21-P23 resulted in a lack of protein expression on WB [20]. No protein expression was detected in patients with the novel homozygous mutations p. S1506X (P25, P26) and p.R182X (P27), while the heterozygous carriers expressed an LRBA protein (approximately 130 kD) at reduced levels compared to healthy controls (Fig. 1g). The healthy controls expressed LRBA proteins of varied sizes while the heterozygous carries only expressed a single-sized protein. For the novel mutation p.N988fs*7 (P29), additional samples could not be obtained for WB but protein expression is predicted to be absent, as for p.Q678X (P15) and p.S1506X (P25, P26), which occur upstream and downstream of p.N988fs*7 respectively, both resulting in absent LRBA expression. The c.4729+2insA

splice site mutation (P30, P31) also resulted in loss of protein expression (Fig. 1g). The clinical phenotypes described here are similar to the spectrum of phenotypes recently described in nine patients with mutations located throughout the LRBA protein and also resulting diminished or loss of expression of the protein [10].

Pathophysiology of LRBA Deficiency

In normal T-cells, LRBA co-localizes with CTLA4 within recycling endosomes and the trans-Golgi network [10]. The transferrin receptor (CD71), known to traffic through recycling endosomes, was also found to be reduced in LRBA knockdown cells. This finding, together with the observation that other proteins (CD28, ICOS, PD-1, CD154) that are known to traffic through other intracellular vesicles are not affected by *LRBA* knockout, suggests a specific role of LRBA in regulating recycling of endosomes [10]. In addition, the colocalization of CTLA4 and LRBA within re-cycling endosomes has been attributed to interaction of the cytoplasmic tail of CTLA4 with the concavalin A-like lectin and PH-BEACH domains of LRBA [10].

Recent finding in patients treated with a CTLA4 –immunoglobulin fusion drug, abatacept, suggest a link between LRBA and CTLA4 [10]. There is a decrease in both the total amount of intracellular and cell-surface CTLA4 in LRBA deficiency patients. The normal *CTLA4* mRNA levels observed suggests that *LRBA* regulates the expression of CTLA4 at a post-translational level [10]. There appears to be a dose-dependent effect of LRBA on the expression of CTLA4, as patients with the highest levels of residual LRBA also showed the highest residual expression of CTLA4. A rapid loss of CTLA4 protein was also observed in normal T-cells subjected to small interfering RNA (siRNA)-mediated knockdown of *LRBA*, further substantiating a post-translational role of LRBA in CTLA4 expression [10]. These abnormalities relating to CTLA4 might contribute to the pathophysiology of LRBA deficiency, particularly in relation to regulatory T cell (Treg) abnormalities. A decreased proportion of Tregs have been observed in some LRBA deficiency patients [10, 16, 20, 21, 24] and functional analyses identified aberrant and reduced suppressive Treg function [16]. A marked decrease of Treg markers (FOXP3, CD25 and CTLA-4), which are key factors in Treg function was observed [10, 16]. These abnormalities might partially account for the gastrointestinal features reported in LRBA deficiency, especially colitis and IBD-like features that are similar to those observed in IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked) syndrome patients [25]. Indeed, transfer of Treg to colitic mice results in resolution of colitis and a similar therapeutic approach might prove effective in humans [26]. An additional perturbation of subsets of the T-cell compartment

has been observed (significantly increased proportion of circulating follicular helper T-cells and decreased proportion of circulating follicular Treg), fitting with the concept of a regulatory defect in (auto)-antibody production [16]. Increased staining of T-cells and Tregs with Annexin V, indicating increased apoptosis, lead to the detection of decreased phosphorylation among downstream substrates of mTORC-1 (mammalian targets of rapamycin complex-1) and decreased activity of mTORC-1 and mTORC-2 kinases [16]. The impaired activation of these mTORC metabolic sensor proteins, could potentially lead to detrimental effects on T-cell function [16].

A functional defect in B-cells or plasma cells may account for the antibody deficiency, as defective/increased apoptosis, decreased survival and reduced autophagy have been observed in B-cells from LRBA-deficient patients [2]. When LRBA-deficient B-cells were cultured (stimulated with anti-CD40/IL-4 and anti-CD40/IL-21) to induce class-switch recombination and maturation into plasmablasts, they failed to proliferate, indicating a defective B-cell differentiation [2]. The low proportions of switched memory B-cells observed in LRBA deficiency (as in CTLA4 haploinsufficiency) might account for the hypogammaglobulinemia observed. However, a B-cell defect alone cannot account for the development of autoimmune diseases observed in patients with LRBA deficiency.

Autophagy, a cellular process facilitating protein and organelle degradation, is important for antigen presentation, plasma cell development and immunological memory [27–29]. B-cells from LRBA deficient patients show reduced autophagy [17]. In the process of B cell activation, the consumption of the SQSTM1 autophagy substrate and the increased expression of autophagy-related Atg proteins suggest that autophagy contributes to plasma cell development [29]. The role that LRBA plays in this process is unknown, however the resulting impairment of autophagy might results in defective B-cell/plasma cell function. BDCPs in general are thought to have a role in membrane dynamics (including both fission and fusion events) and in endosomal, lysosomal and vesicular trafficking [6]. The BDCP, WDFY3 (Table 1), forms a heterodimer with SQSTM1 via binding of its PH and BEACH domains that acts as a scaffold linking ubiquitinated cargos to autophagosomes, facilitating autophagy [6]. Other roles of autophagy relate to homeostasis of the endoplasmic reticulum (ER), energy metabolism and stress responses, thereby controlling secretion of immunoglobulins [29]. In vivo, plasma cells from an autophagy deficient mouse model showed reduced levels of adenosine-triphosphate (ATP) compared to wild type cells, as well as shorter life-span [29]. In addition, mice with reduced rate of autophagy showed fewer and smaller germinal centers than controls [30]. It is hypothesized that autophagy plays an essential role in limiting the stressful metabolic conditions

of immunoglobulin synthesis to sustainable levels while simultaneously optimizing energy metabolism.

The mechanism by which the antibody deficiency occurs may be due to a combination of defective autophagy, increased apoptosis and a subsequent arrested proliferation of B-cells/plasma cells. Linking the autophagy defect to perturbation of intestinal mucosal homeostasis and the gastrointestinal disorders observed in LRBA-deficient patients might be compatible with the increased risk of developing IBD (Crohn's disease) in patients with mutations in genes that are involved in the autophagy process [27] and disruption of the autophagosomal processing of intracellular bacteria in mucosal cells of LRBA-deficient patients might relate to the 'inflammatory barrier disorder' concept of Crohn's disease [31].

The additional mutations that were identified in some patients using WES (Supplementary Table 5) are unlikely to contribute to the clinical features of LRBA deficiency as all were heterozygous mutations. However, yet unidentified modifier genes might be contributing to the different phenotypes observed in the patients. We suggest that a search for genes related to CVID-like disease, IBD-like disease and autoimmune disorders could be included in further investigation of cases with LRBA deficiency.

LRBA deficiency and CTLA4-deficiency appear to share clinical features including the development of autoimmune disorders (e.g., autoimmune cytopenias), hypogammaglobulinemia, chronic diarrhea, respiratory tract infections and organomegaly [10, 32, 33]. Lymphocytic infiltration of non-lymphoid organs also appears to be a feature of both disorders. In contrast to CTLA4 deficiency, almost all patients with LRBA deficiency manifest clinically [32, 33]. Suppression of Treg function occurs in both disorders, which might account for the shared features of autoimmunity.

Conclusion

Mutations affecting any exons or segments of *LRBA* result in a spectrum of clinical phenotypes including chronic diarrhea, autoimmune disorders, organomegaly, hypogammaglobulinemia, respiratory infections or combinations of these phenotypes. A propensity for serious neurological complications appears to be present as well. LRBA deficiency is surprisingly common among patients suffering from a CVID-like phenotype. The regulatory T-cell compartment is compromised and switched-memory B-cells are deficient in most cases. Screening for mutations in *LRBA* or for the protein may thus be of value in patients suffering from the above mentioned phenotypes, and should also be considered in CVID and CVID-like disorders. Abatacept may potentially be a therapeutic option in cases of LRBA deficiency. However, the precise role that LRBA plays in the pathogenesis of these disorders needs to be investigated further.

Contribution O.K. and H.A. performed experiments, analyzed results, made the figures, and wrote the paper; M.F. analyzed results; K.K., M.M., and J.C. performed experiments and analyzed results; H.A., Z.C., I.M., and M.E. performed clinical sampling and; A.A., N.R., R.G. and L.H. designed the research and edited the paper.

Compliance with Ethical Standards

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Conflict of Interest The authors declare no competing financial interests.

Multiple Choice Questions

Somatic mutations in *LRBA* have been linked to:

1. Chronic diarrhea
2. Immune thrombocytopenic purpura
3. Autoimmune hemolytic anemia
4. Breast cancer
5. Hypogammaglobulinemia

In LRBA deficiency, B-cell characteristics most commonly include:

1. An increased proportion of switched memory B-cells
2. A decreased proportion of switched memory B-cells
3. Normal autophagic function
4. Normal B-cell proliferation
5. Normal B-cell differentiation

Regarding mutations resulting in *LRBA*, which of the following statements is correct:

1. Only fragment deletions result in LRBA deficiency
2. Only nonsense mutations result in LRBA deficiency
3. Only mutations in exon 30 result in LRBA deficiency
4. Multiple mutations are necessary to result in LRBA deficiency
5. A single homozygous mutation may result in LRBA deficiency

LRBA is a:

1. Cell-surface receptor protein
2. Expressed in lymphoid cells only
3. Expressed in neural cells only
4. Expressed in B-cells only
5. Cytosolic protein

Regarding plasma cells, which of the following is correct :

1. Do not have autophagic activity
2. Have high autophagic activity

3. Lose their endoplasmic reticula during differentiation
4. Do not express autophagy-related proteins
5. Have a very low level of autophagy substrate SQSTM1 protein

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