

# Normal Expression of Cytotoxic T-lymphocyte-Associated Protein4 (CTLA4) in a Lipopolysaccharide-Responsive and Beige-like Anchor Protein (LRBA) Deficient Patient

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## Abstract

Biallelic lipopolysaccharide-responsive and beige-like anchor (LRBA) mutations could lead to an immune dysregulation disorder, labeled as LRBA deficiency. A wide spectrum of clinical manifestation was shown to be associated with recurrent infections, enteropathy, hypogammaglobulinemia, and autoimmune complications. Notably, LRBA interacts with cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) by its recycling to the T-cell surface. Accordingly, LRBA deficiency abolishes CTLA4 protein expression. In this study, we presented a case with a homozygous mutation in the *LRBA* gene as well as a normal level of CTLA4 protein. In this regard, the immunologist assays of this patient revealed low immunoglobulin levels, CD4<sup>+</sup> helper T cells, and CD19<sup>+</sup> B cells.

**Keywords:** LRBA, hypogammaglobulinemia, enteropathy, CTLA4

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## Archive of SID Introduction

Lipopolysaccharide-responsive and beige-like anchor (LRBA) deficiency is known as the predominant immune dysregulation induced by bi-allelic loss-of-function mutations in the LRBA gene (which encodes lipopolysaccharide-responsive and beige-like anchor protein) that often abolishes LRBA expression. Moreover, LRBA deficiency causes extensive clinical phenotypes such as autoimmunity, hypogammaglobulinemia, and other forms of humoral immune deficiency, lymphoproliferation, gastrointestinal manifestations, and recurrent infections (1-4). Correspondingly, autoimmune disorders are considered as one of the main clinical manifestations' characteristics in LRBA deficiency. It is noteworthy that Genotype-phenotype correlation is just documented in less than 100 patients reported with LRBA deficiency (1).

Also, LRBA deficiency causes some immunological abnormalities such as low immunoglobulin G (IgG) and IgA levels, whereas IgM levels can be low or normal during the course of this disease. Several patients with LRBA deficiency have been reported with defects in specific antibody responses, deficiency in the B-cell compartment with the reduced numbers of switched memory B cells and plasmablasts, and diminished T-cells activation and proliferation (5-7). The majority of LRBA deficient patients indicate B-cell abnormalities, such as the increased number of CD21<sup>low</sup> B cells and marginal zone B cells. In addition, the increased apoptosis and decreased autophagy in B lymphocytes as well as natural killer-(NK) cell reduction were also documented in LRBA deficient patients (6, 8, 9).

CTLA-4, also known as CD152 (cluster of differentiation 152), is a protein receptor that functions as an immune checkpoint and downregulates immune response. Moreover, it is expressed on forkhead box P3 (FOXP3)<sup>+</sup> regulatory T (Treg) cells (10). In this regard, heterozygous CTLA4 mutations cause functional CTLA-4 deficiency and autoimmunity, both of which present a sim-

ilar clinical signature of LRBA deficiency (11). It has been shown that LRBA protein regulates the intracellular vesicle trafficking of CTLA4 and also prevents it from lysosomal degradation; therefore, LRBA deficiency can hypothetically lead to the diminished expression of CTLA-4 on the surface of Treg cells (12, 13).

Herein, we presented an LRBA deficient patient with defined molecular diagnosis and abolished LRBA protein expression, which in contrast, could express a normal level of CTLA-4.

## Case Presentation

The patient was a 17-year-old girl born from consanguineous healthy parents. Accordingly, she was the first child, and her family had no history of immunologic medical problems. When she was 7 months old, her first manifestation was diarrhea, which consequently resulted in 5 days of hospitalization. According to the patients' past medical history, at the age of 5 years old, she was suffering from otitis media, dry cough, juvenile rheumatoid arthritis (JRA), hepatosplenomegaly, and bronchiectasis. In addition, mild chronic gastritis, mild esophagitis, and acute colitis were also reported when she was 12 years old.

Data on the laboratory and immunological tests for the proband are shown in **Table 1**. As indicated in this table, the patient presented normal white blood cells (WBC) differentiation; however, the percentages of neutrophils and lymphocytes were normal. Despite lymphocytes, the percentage of immunologic cells was reported as low. Serum immunoglobulin levels were also tested, which revealed low levels of IgG, IgM, and IgA. The immunological workup showed a decrease in CD4<sup>+</sup> T cells and CD19<sup>+</sup> B cells levels. Moreover, normal levels of erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were detected in this patient. Subsequently, she has been treated with Prednisolone, Hydroxychloroquine, and Infliximab (for a 5-month period).

Table 1. Laboratory and immunologic data of the patient

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Laboratory test	Patient	Reference Intervals
WBC ( $10^3/\mu\text{l}$ )	12	4.5-13.5
Lymph (%)	56	34-77
PMN (%)	39	35-75
Hb (gr/dl)	12.7	9.4-15.5
Hematocrit (%)	39	34-45
PLT ( $10^3/\mu\text{l}$ )	312	150-450
ESR 1 hr (mm/hr)	2	<20
CRP (mg/L)	4.7	Up to 10
IgG (mg/dl)	444	650-1410
IgM (mg/dl)	<5	55-210
IgA (mg/dl)	4	83-255
IgE (IU/ml)	1	Up to 15
CD3+ T cells (%)	73	59-83
CD4+ T cells (%)	4	31-59
CD8+ T cells (%)	66	12-38
CD16+ NK cells (%)	7	3-22
CD19+ B cells (%)	3.5	6-22

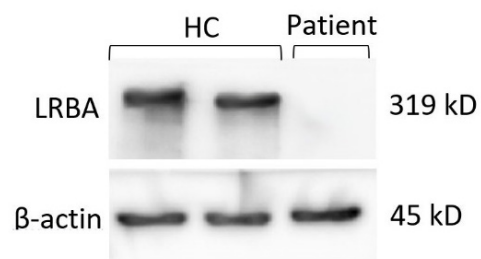
WBC, white blood cell; Lymph, lymphocytes; PMN, Polymorphonuclear leukocyte; PLT, platelet; Hb, hemoglobin; ESR, Erythrocyte sedimentation rate; CRP, C-reactive Protein; Ig, immunoglobulin; and CD, cluster of differentiation

Based on the clinical presentations of immune dysregulation and the immunologic profile of the patient resembling humoral immunodeficiency, the genetic evaluation was performed using

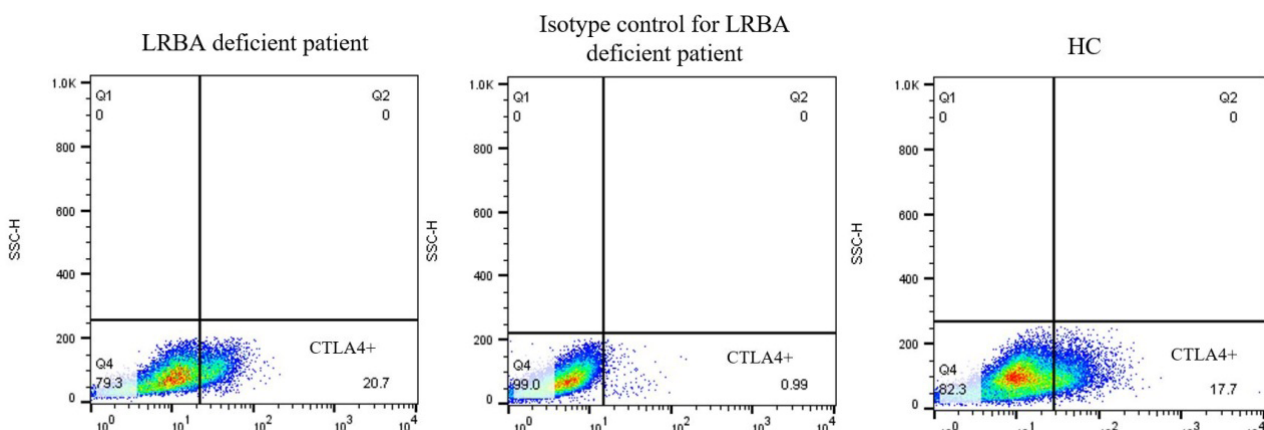
whole-exome sequencing (WES). Furthermore, the WES that was analyzed using the previously developed pipeline (14, 15), demonstrated a homozygous frame-shift deletion mutation (c.5623delA) in the *LRBA* gene (p.I1875SfsX14).

A functional assay was conducted to evaluate the expression level of the encoded protein. Expectedly, using Western blot on stimulated peripheral blood cells (PBMC), the mutant LRBA protein (estimated 319 kDa) was undetectable compared to the healthy controls (**Figure 1**). CTLA-4 proteins on the surface of stimulated PBMCs were assessed by flow cytometry. Notably, the LRBA deficient patient had a normal level of CTLA4 expression, similar to the healthy controls. (**Figure 2**)

**Figure 1.** Detection of LRBA protein (~319 kDa) and  $\beta$ -actin (~45 kDa) in PHA-stimulated cell lysates from HCs of the patient, which revealed the absence of the LRBA protein in PHA-stimulated cell lysate from this patient with LRBA deficiency, compared to the LRBA band observed in PHA-stimulated cell lysates obtained from healthy controls.  $\beta$ -actin served as the loading control.



**Figure 2.** Approach for the stimulated lymphocyte gating in LRBA deficient patient and healthy controls for the recognition of CTLA-4<sup>+</sup> lymphocytes.



## Archive of SID Discussion

Homozygous mutation in the *LRBA* gene resulted in a heterogeneous group of disorders, each one with a broad spectrum of clinical and immunological features such as autoimmunity, enteropathy, organomegaly, hypogammaglobulinemia, and recurrent infections (6, 8, 16, and 17). *LRBA* was colocalized with CTLA4 in endosomes, which then mediated CTLA-4 recycling from the endosomes to the T-cell surface and prevented lysosomal degradation of CTLA4. Afterward, *LRBA*-deficient cells displayed low CTLA-4 expression levels in FOXP3<sup>+</sup> regulatory and activated conventional T cells (18, 19).

Laura Gamez-Diaz et al., in their cohort study in 2015, have described 22 *LRBA*-deficient patients. In this regard, they reported a mutation in the *LRBA* gene, causing hypogammaglobulinemia, organomegaly, autoimmunity, enteropathy, and recurrent infections (20). In the review study by Alkheiry et al. (5), the clinical features were observed in 31 patients with *LRBA* deficiency. In their study, 61% of the patients had chronic diarrhea, autoimmune disease, organomegaly, and respiratory infections separately, and 58% of patients with *LRBA* deficiency had hypogammaglobulinemia. Moreover, Tesi et al. presented a case of *LRBA* deficiency with low levels of IgG, IgM, and IgA and the need for intravenous immunoglobulin (IVIG) (21). Furthermore, several other studies have reported low levels of serum IgG, IgA, and IgM in *LRBA* deficiency (1, 20). In Azizi et al.'s case report performed on two male siblings with the same mutation, one patient demonstrated clinical phenotypes including hypogammaglobulinemia, chronic diarrhea, respiratory tract infection, and organomegaly; however, the other sibling had no clinical complications and showed a normal serum Ig level (3). Similarly, in our research, autoimmunity, recurrent infections, enteropathy, autoimmunity, and hepatosplenomegaly were documented in the patient studies. In this case, hypogammaglobulinemia was indicated by low levels of IgG, IgA, and IgM.

It was shown that homozygous *LRBA* mutations might lead to deficiencies in CD19<sup>+</sup> B cell, CD4<sup>+</sup> T cell, and NK cell (9). Moreover, as we have observed in the proband, some previously reported patients with *LRBA* deficiency have shown low B-cell subset counts, defective specific antibody production, and a defect in the frequency and function of CD4<sup>+</sup> T cells compared with those of healthy controls (7).

Habibi et al. have reported 74 *LRBA*-deficient patients, the majority of whom suffered from reduced numbers of CD4<sup>+</sup> and CD8<sup>+</sup> T cells. Treg cells counts in the peripheral blood cells of these patients had also decreased. In addition, the frequency of NK cells had reduced. Furthermore, they had shown that CD21<sup>low</sup> B cells counts have increased, and plasmablast counts and Switched memory B cells have reduced (1). Similarly, laboratory findings in our patient showed a significant decrease in CD19<sup>+</sup> B cells and CD4<sup>+</sup> T cells. Moreover, she demonstrated low B cell subset counts (switched memory B cells and plasmablasts).

Lo et al., in their study, showed that CTLA4 protein has rapidly lost in *LRBA* knockdown cells, indicating an increase in degradation (16). Recently, few studies have reported a lack of CTLA4 expression in patients with *LRBA* gene mutation. It was expected that *LRBA* deficiency consequently leads to functional CTLA-4 deficiency and autoimmunity, based on the physiologic role of the protein. However, *LRBA* had been reported as a novel regulator of CTLA-4 protein levels in Tregs and activated T cells (12, 16, 18, 22, 23). Whereas, in this study, we detected a normal level of CTLA4 in *LRBA* deficient patients with a deleterious frameshift mutation. Similarly, in another study, De Bruyne et al. (24), for the first time, had reported a girl born from a consanguineous marriage which had life-threatening enteropathy. Immunological features of this patient revealed normal serum immunoglobulins. Moreover, the patient presented a well-developed switched memory B-cell compartment.



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In their study, CD4<sup>+</sup> T cells were normal, whereas CD19<sup>+</sup> B cells have persistently increased. They suggested that other proteins such as the Beige and Chediak-Higashi (BEACH) domain-containing protein [a member of the neurobeachin (NBEA) family] could conceivably be taken over CTLA-4 recycling, which is required for the function of Treg cells, and/or treatment with Sirolimus, which increased Treg cells, may be considered as the fundamental causes for the condition of this patient. It is noteworthy that their case was affected by a homozygous splice site mutation (c.2450-3C>A), which caused deleterious damage with the skipping of exon 21 as well as a frameshift resulting in a premature stop codon in exon 22 after 29 residues, similar to our case. Altogether, these findings show that mutation in the *LRBA* gene could be revealed by unexpected features for patients with immunological abnormalities. However, the compensatory mechanism for CTLA4 expression should still be investigated.

### Conclusion

In the present study, for the first time, we reported a girl with a mutation in the *LRBA* gene and a normal CTLA4 who showed significantly low levels of CD4<sup>+</sup> T cells and CD19<sup>+</sup> B cells, as well as normal lymphocyte counts, along with otitis media, bronchiectasis, arthritis, and hepatosplenomegaly. Our data showed that *LRBA* mutation may not always reduce CTLA4 protein. Additionally, autoimmunity can be observed in a patient with a normal level of CTLA4 protein. Also, wide heterogeneity was observed in clinical manifestations of patients with *LRBA* mutation.

### Conflict of Interest

The authors declare no conflicts of interest.

### Acknowledgment

The patient's legal guardians signed a written informed consent for the publication of this case report and its accompanying information.

### References

1. Habibi S, Zaki-Dizaji M, Rafiemanesh H, Lo B, Jamee M, Gamez-Diaz L, et al. Clinical, Immunologic, and Molecular Spectrum of Patients with LPS-Responsive Beige-Like Anchor Protein Deficiency: A Systematic Review. *J Allergy Clin Immunol Pract*. 2019;7(7):2379-86 e5.
2. Azizi G, Abolhassani H, Zaki-Dizaji M, Habibi S, Mohammadi H, Shaghaghi M, et al. Polyautoimmunity in Patients with LPS-Responsive Beige-Like Anchor (*LRBA*) Deficiency. *Immunol Invest*. 2018;47(5):457-67.
3. Azizi G, Abolhassani H, Habibi S, Rahamooz T, Mohammadi H, Jafarnezhad-Ansariha F, et al. Two Faces of *LRBA* Deficiency in Siblings: Hypogammaglobulinemia and Normal Immunoglobulin Levels. *J Invest Allergol Clin Immunol*. 2018;28(1):48-50.
4. Azizi G, Abolhassani H, Yazdani R, Mohammadikhajehdehi S, Parvaneh N, Negahdari B, 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.
5. Alkhairy OK, Abolhassani H, Rezaei N, Fang M, Andersen KK, Chavoshzadeh Z, et al. Spectrum of Phenotypes Associated with Mutations in *LRBA*. *J Clin Immunol*. 2016;36(1):33-45.
6. Lopez-Herrera G, Tampella G, Pan-Hammarstrom Q, Herholz P, Trujillo-Vargas CM, Phadwal K, 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.
7. Azizi G, Mirshafiey A, Abolhassani H, Yazdani R, Ghanavatinejad A, Noorbakhsh F, et al. The imbalance of circulating T helper subsets and regulatory T cells in patients with *LRBA* deficiency: Correlation with disease severity. *J Cell Physiol*. 2018;233(11):8767-77.
8. Salami F, Shirkani A, Shahrooei M, Azizi G, Yazdani R, Abolhassani H, et al. Leishmaniasis and Autoimmunity in Patient with LPS-Res-

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- sponsive Beige-Like Anchor Protein (LRBA) Deficiency. *Endocr Metab Immune Disord Drug Targets*. 2020;20(3):479-84.
9. Azizi G, Abolhassani H, Mahdavian SA, Chavoshzadeh Z, Eshghi P, Yazdani R, et al. Clinical, immunologic, molecular analyses and outcomes of iranian patients with LRBA deficiency: A longitudinal study. *Pediatr Allergy Immunol*. 2017;28(5):478-84.
  10. Rowshanravan B, Halliday N, Sansom DM. CTLA-4: a moving target in immunotherapy. *Blood*. 2018;131(1):58-67.
  11. Lo B, Abdel-Motal UM. Lessons from CTLA-4 deficiency and checkpoint inhibition. *Curr Opin Immunol*. 2017;49:14-9.
  12. Alroqi FJ, Charbonnier LM, Baris S, Kiykim A, Chou J, Platt CD, 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):1050-9 e10.
  13. Košťel Bal S, Haskologlu S, Serwas NK, Islamoglu C, Aytakin C, Kendirli T, et al. Multiple Presentations of LRBA Deficiency: a Single-Center Experience. *J Clin Immunol*. 2017;37(8):790-800.
  14. Lo B, Zhang K, Lu W, Zheng L, Zhang Q, Kanellopoulou C, et al. Autoimmune Disease. Patients with LRBA deficiency show CTLA4 loss and immune dysregulation responsive to abatacept therapy. *Science*. 2015;349(6246):436-40.
  15. Martínez Jaramillo C, Trujillo-Vargas CM. LRBA in the endomembrane system. *Colomb Med (Cali)*. 2018;49(3):236-43.
  16. Cepika AM, Sato Y, Liu JM, Uyeda MJ, Bacchetta R, Roncarolo MG. Tregopathies: Monogenic diseases resulting in regulatory T-cell deficiency. *J Allergy Clin Immunol*. 2018;142(6):1679-95.
  17. De Bruyne M, Bogaert DJ, Venken K, Van den Bossche L, Bonroy C, Roels L, et al. A novel LPS-responsive beige-like anchor protein (LRBA) mutation presents with normal cytotoxic T lymphocyte-associated protein 4 (CTLA-4) and overactive T(H)17 immunity. *J Allergy Clin Immunol*. 2018;142(6):1968-71.
  18. Gámez-Díaz L, August D, Stepensky P, Revel-Vilk S, Seidel MG, Noriko M, et al. The extended phenotype of LPS-responsive beige-like anchor protein (LRBA) deficiency. *J Allergy Clin Immunol*. 2016;137(1):223-30.
  19. Tesi B, Priftakis P, Lindgren F, Chiang SC, Kartalis N, Löfstedt A, et al. Successful Hematopoietic Stem Cell Transplantation in a Patient with LPS-Responsive Beige-Like Anchor (LRBA) Gene Mutation. *J Clin Immunol*. 2016;36(5):480-9.
  20. Azizi G, Jamee M, Yazdani R, Bagheri Y, Fayyaz F, Jadidi-Niaragh F, et al. CTLA-4 Expression in CD4+ T Cells From Patients With LRBA Deficiency and Common Variable Immunodeficiency With No Known Monogenic Disease. *J Investig Allergol Clin Immunol*. 2018;28(6):422-4.
  21. Burnett DL, Parish IA, Masle-Farquhar E, Brink R, Goodnow CC. Murine LRBA deficiency causes CTLA-4 deficiency in Tregs without progression to immune dysregulation. *Immunol Cell Biol*. 2017;95(9):775-88.
  22. De Bruyne M, Bogaert DJ, Venken K, Van den Bossche L, Bonroy C, Roels L, et al. A novel LPS-responsive beige-like anchor protein (LRBA) mutation presents with normal cytotoxic T lymphocyte-associated protein 4 (CTLA-4) and overactive TH17 immunity. *J Allergy Clin Immunol*. 2018;142(6):1968-71.
  23. Burnett DL, Parish IA, Masle-Farquhar E, Brink R, Goodnow CC. Murine LRBA deficiency causes CTLA-4 deficiency in Tregs without progression to immune dysregulation. *Immunol Cell Biol* 2017;95(9):775-88.
  24. De Bruyne M, Bogaert DJ, Venken K, Van den Bossche L, Bonroy C, Roels L, et al. A novel LPS-responsive beige-like anchor protein (LRBA) mutation presents with normal cytotoxic T lymphocyte-associated protein 4 (CTLA-4) and overactive TH17 immunity. *J Allergy Clin Immunol* . 2018; 142(6):1968-71.