

Letter to the Editor

LRBA gene deletion in a patient presenting with autoimmunity without hypogammaglobulinemia

To the Editor:

Primary immunodeficiencies are a highly heterogeneous group of genetic disorders caused by Mendelian mutations in >150 immune-related genes.¹ Primary immunodeficiencies manifest as severe and/or disseminated recurrent infections and may also have autoimmune manifestations.

We studied a female patient P1 of Pakistani origin who presented at the age of 4 years with a generalized lymphadenopathy, splenomegaly, neutropenia (range, $0.05\text{--}0.28 \times 10^9/\text{L}$), and thrombocytopenia (platelet count, $20\text{--}40 \times 10^9/\text{L}$). A lymph node biopsy showed reactive changes, bone marrow aspirate was unremarkable, and antineutrophil antibodies were present. She also had chronic diarrhea associated with an autoimmune enteropathy, characterized by duodenal villous atrophy and large bowel lymphocytic infiltration on biopsy. Her initial immunology workup found only raised IgG levels (22.6 g/L), raised inflammatory markers, and a low number of natural killer cells (0.00–0.02). Lymphocyte subsets, double-negative T cells, T-cell proliferation assays, IgA (1.33 g/L), IgM (1.43 g/L), tetanus vaccine responses, and a nitroblue tetrazolium test were normal (Table I). P1 had no significant history of infections except for a psoas abscess associated with chronic neutropenia. Over time she manifested growth

failure and developed new autoimmune features, including an episode of erythema nodosum, transient arthritis of both feet, and recurrent hemolytic anemia. As a result, she received several courses of steroids, rituximab (with prophylactic immunoglobulin replacement), and mycophenolate mofetil. Five years after initial presentation, after multiple courses of rituximab, she developed recurrent infections (*Streptococcus pneumoniae* facial cellulitis, *Streptococcus pneumoniae* sepsis, and *Haemophilus influenzae* empyema) after withdrawal of immunoglobulin therapy. Although both her CD19⁺ B cells and IgG level were normal, further investigation found a new-onset antibody deficiency with absent vaccine responses (Table I). Because of further chest symptoms, despite recommencing immunoglobulin replacement, a chest computed tomographic scan was performed that showed extensive lung infiltration. Lung biopsy showed a florid diffuse lymphoid interstitial infiltrate that consisted of a mixture of CD3⁺ T and CD20⁺ B cells, with scattered lymphoid follicles, particularly around airways. No granulomata were seen, and stains for bacteria, fungi, and mycobacteria, as well as *in situ* hybridisation for EBV, were negative.

Patient P1 was born to a consanguineous marriage of first cousins. Therefore, we hypothesized that her disease was caused by a homozygous mutation. To identify the causative mutation we used exome sequencing. Patient's blood sample was obtained with informed consent from the parents in accordance with the

TABLE I. Serial immunology assessments

Age (y)	Steroid	Rituximab	Other	Ig	Infections	Lymph*	CD3 ⁺ T cells*	CD4 ⁺ T cells*	CD8 ⁺ T cells*	NK cells*	CD19 ⁺ B cells*	Naive B cells†	CSM B cells†	IgG‡	IgA‡	IgM‡	Tet§	PA
4.8	No			No		2.45	1.64	1.2	0.49	0.01↓	0.49	ND	ND	22.6↑	1.33	1.43	1.21	ND
5.0	No			No	Sterile psoas abscess													
5.3	Yes	First course		Yes														
5.9	No		Aza	Yes		2.06	2	1.13	0.8	0↓	0↓			8.29	0.45↓	0.26↓		
7.5	No			No		2.47	1.83	1.11	0.69	0.07↓	0.52	82	3↓	4.02↓	1.49	6.81↑	0.7	5/13¶
7.7	Yes	Second course		Yes														
7.8	Yes			Yes		1.79	1.7	1.06	0.61	0.07↓	0↓			7.03	0.48	0.94		
8.0	No		MMF	Yes		1.78	1.46	0.87	0.53	0.11	0.14↓			9.73	0.2↓	0.31↓		
9.0	No		MMF	No	Pneumococcal sepsis			1.42	0.54	0.08↓	0.36			7.21	0.16↓	3.65↑	0.57	0/13¶
9.3	No		MMF	No	Pneumococcal facial cellulitis, Hib empyema	2.99	2.36	1.67	0.63	0.06↓	0.45			6.01	0.11↓	5.96↑		0/13
9.7	Yes	Third course	MMF	Yes														
9.9	No		MMF	Yes		1	0.88	0.55	0.31	0.09	0↓			7.02	0.12↓	1.87		
10.2	No		MMF	Yes		1.55	1.35	0.93	0.36	0.08↓	0.09↓	96↑	0.4↓	13.4	0.09↓	12.1↑		
10.4	Yes		MMF	Yes		1.24	0.88	0.53	0.32	0.04↓	0.27			10.3	0.09↓	5.89↑		

Concomitant immunosuppression and immunoglobulin replacement are shown. Infections are paired with the first set of results after the infectious episode. The patient received tetanus vaccination before first analysis as part of routine childhood vaccination. Pneumococcus vaccine was administered for the first time at the age of 9.

Aza, Azathioprine; CSM, class-switched memory; Hib, *Haemophilus influenzae*; Ig, immunoglobulin replacement; Lymph, absolute lymphocyte count; MMF, mycophenolate mofetil; ND, not done; NK, natural killer; PA, pneumococcus antibodies (no. of positive response to 13 serotypes measured); Tet, tetanus antibodies; ↓ or ↑ denotes results below or above normal range for age, respectively.

*Units are $\times 10^9/\text{L}$.

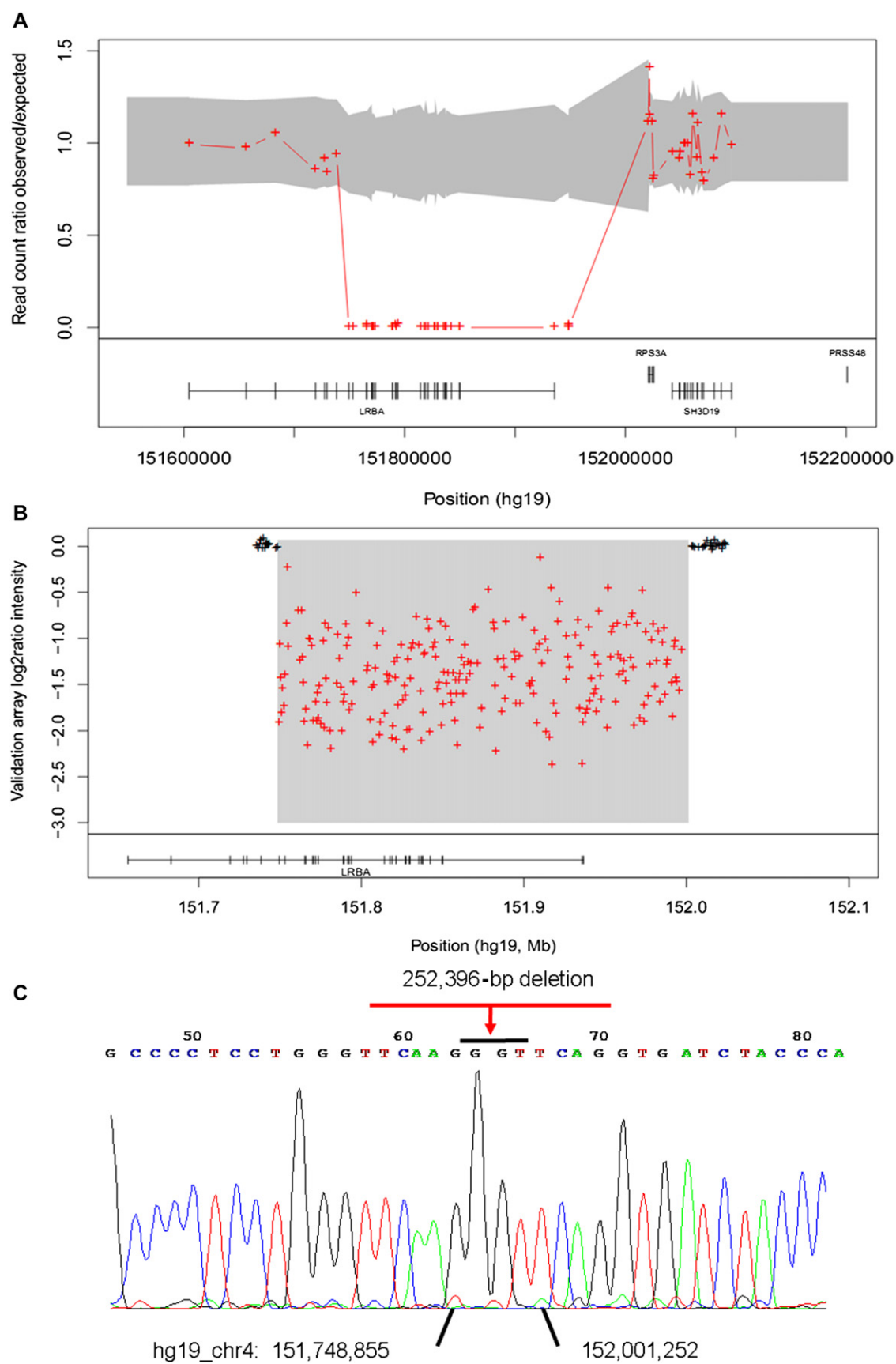
†Naive (IgD⁺CD27⁻) and class-switched memory (IgD⁻CD27⁺) B cells are shown as a percentage of CD19⁺ B cells.

‡Units are g/L.

§Units are IU/ml, where >0.1 is protective.

||After vaccination with 7 valent conjugate pneumococcal vaccine.

¶Before vaccination.



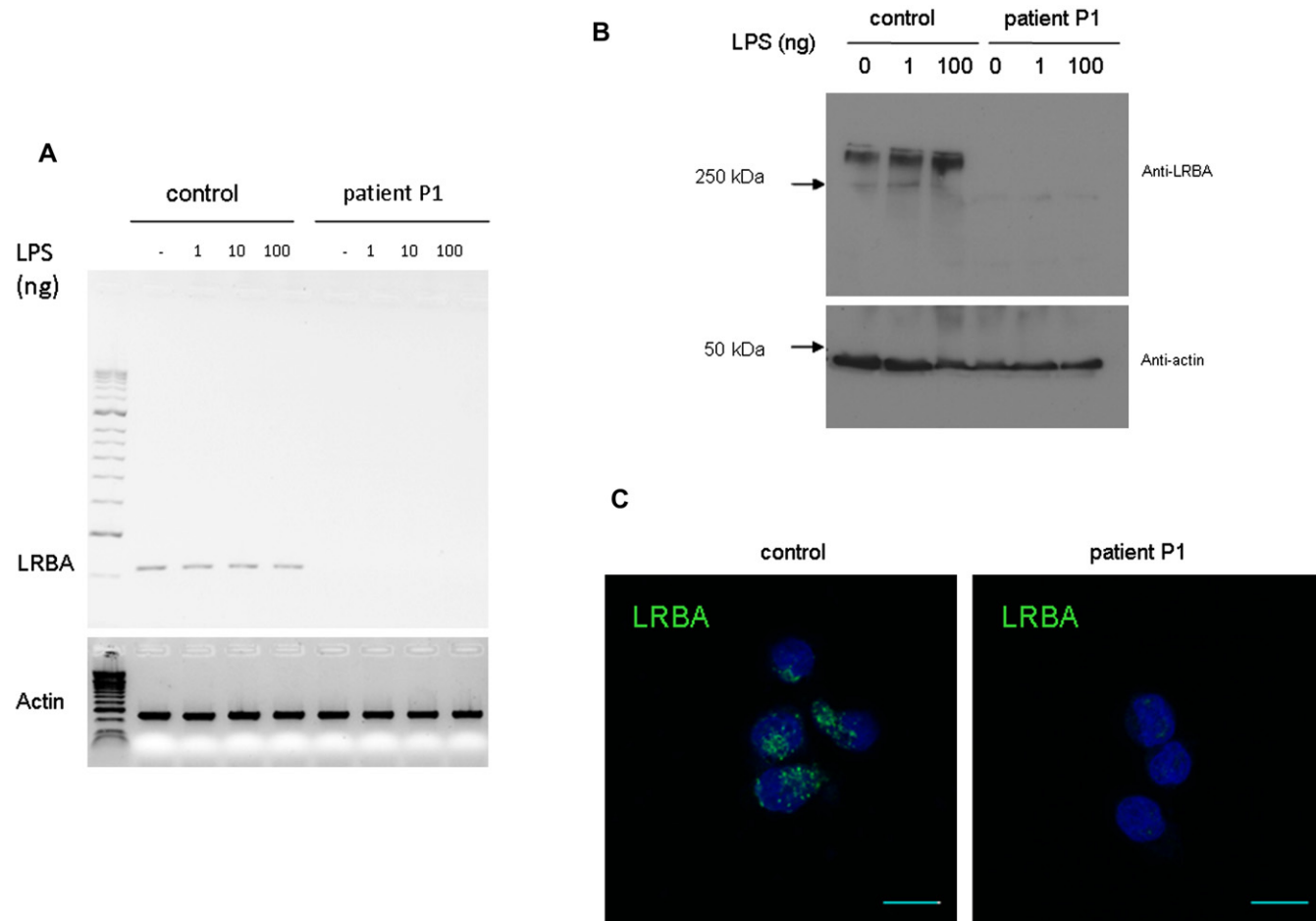


FIG 2. LRBA mRNA and protein are expressed in the EBV-transformed B-cell line from a healthy control but are absent in patient P1. **A**, RT-PCR. **B**, Western blot analysis. **C**, Immunofluorescence analysis (LRBA, green; DNA, blue; scale bars, 10 μ m).

Declaration of Helsinki and with approval from the ethics committees (04/Q0501/119 and 06/Q0508/16). Library preparation, exome capture, and sequencing were done according to the manufacturer's instructions. For exome target enrichment the Agilent SureSelect 38 Mb kit was used. Sequencing was done with Illumina HiSeq with 94-bp paired-end reads. In the exome data we found 17,280 single nucleotide variants and small insertions/deletions, including 424 rare ones, that is, those not seen in the 1000 Genomes database (May 2011 data release). However, we found no homozygous loss-of-function mutations. To identify larger deletions and duplications we used a new software tool, ExomeDepth (available to download at <http://cran.r-project.org/web/packages/ExomeDepth/index.html>; V. Plagnol, et al, 2012). We found a large homozygous deletion that removed exons 1 to 30 of the 58-exon LPS-responsive vesicle trafficking, beach, and anchor containing (*LRBA*) gene (Fig 1, A). This deletion is novel and not present in the Database of Genomic Variants (<http://projects.tcag.ca/variation/>). We validated it with

the use of a custom comparative genomic hybridization array that contained 270 probes in a 288-kb region defined around the *LRBA* gene (Fig 1, B). We then sequenced the exact boundaries of this 252,396-nucleotide deletion (chr4: 151,748,856-152,001,251; Fig 1, C).

We established an EBV-transformed B-cell line of the patient and studied LRBA expression. We stimulated cells with 0, 1, 10, or 100 ng/mL LPS for 16 hours. Then we extracted RNA, generated cDNA, and amplified a part of the *LRBA* gene with the use of primers 5'-GCAGAAGTCATGCTTGGACA-3' and 5'-TTTCGAAGTAGGGTCGCAAT-3'. An expected 218-bp product from the *LRBA* exons 19 to 21 was present in control, but not in patient P1 (Fig 2, A). From the LPS-stimulated cells we also extracted proteins and separated them with the use of SDS-PAGE. For Western blot analysis we used anti-LRBA (Sigma HPA019366) and anti-actin antibodies. We found the LRBA protein with the expected mass of ~319 kDa in control cells but not in cells of patient P1 (Fig 2, B). We also used immunofluorescence

FIG 1. Homozygous deletion in the *LRBA* gene region. **A**, Homozygous deletion identified by ExomeDepth in the exome sequence data (estimated 99% CI is shown in gray). **B**, Validation of ~252-kb deletion with the use of a targeted comparative genomic hybridization array. **C**, Sequencing of the deletion breakpoints (in patient P1 the deleted region is replaced by 3 nucleotides, GGT).

analysis and again found that LRBA protein is expressed in the cytoplasm of cells from a healthy control but not from patient P1 (Fig 2, C).

The *LRBA* gene encodes a large broadly expressed protein of unknown function that is involved in intracellular vesicle trafficking.² Recently, Lopez-Herrera et al³ reported 5 patients with different homozygous mutations in the *LRBA* gene that also abolished its expression. These patients had idiopathic thrombocytopenic purpura, lymphoid interstitial pneumonia, and autoimmune enteropathy, as well as hypogammaglobulinemia. Reduced levels of IgG and IgA were found in all 5 patients and reduced IgM was recorded in 4 of those patients. Accordingly, all 5 patients have been diagnosed with childhood-onset common variable immunodeficiency. Patient P1 that we report here had many similar clinical features. However, hypogammaglobulinemia was not seen at presentation but occurred as a later event, and it may have been secondary to rituximab therapy, as has previously been described.⁴ Thus, our results show that deficiency caused by mutations in the *LRBA* gene may initially present as an autoimmune syndrome without common variable immunodeficiency. Therefore, a possibility of *LRBA* gene mutations should be considered for a broad spectrum of patients with primary immunodeficiencies and autoimmunity.

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