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LRBA gene deletion in a patient presenting with autoimmunity without hypogammaglobulinemia

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To the Editor:

Primary immunodeficiencies are a highly heterogeneous group of genetic disorders caused by Mendelian mutations in more than 150 immune-related genes¹. 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 ($0.05\text{--}0.28 \times 10^9/\text{L}$) and thrombocytopenia (platelet counts $20\text{--}40 \times 10^9/\text{L}$). A lymph node biopsy showed reactive changes, bone marrow aspirate was unremarkable and anti-neutrophil 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 work up revealed only raised IgG levels (22.6 g/L), raised inflammatory markers and a low number of NK 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 (NBT) test were normal (Table 1). P1 had no

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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 haemolytic anaemia. As a result, she received several courses of steroids, rituximab (with prophylactic immunoglobulin replacement) and mycophenolate mofetil. Five years after initial presentation, following 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 revealed a new-onset antibody deficiency with absent vaccine responses (Table 1). Due to further chest symptoms, despite recommencing immunoglobulin replacement, a chest CT scan was performed that showed extensive lung infiltration. Lung biopsy showed a florid diffuse lymphoid interstitial infiltrate consisting 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 is 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 Declaration of Helsinki and with approval from the ethics committees (04/Q0501/119 and 06/Q0508/16). Library preparation, exome capture and sequencing have been done according to the manufacturers instructions. For exome target enrichment the Agilent SureSelect 38 Mb kit was used. Sequencing was done using 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 very rare ones, i.e. 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, ExomeDepth² (available to download at <http://cran.r-project.org/web/packages/ExomeDepth/index.html>). 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 (Figure 1A). This deletion is novel and not present in the Database of Genomic Variants (DGV, <http://projects.tcag.ca/variation/>). We validated it using a custom comparative genomic hybridization (CGH) array containing 270 probes in a 288 kb region defined around the LRBA gene (Figure 1B). We then sequenced the exact boundaries of this 252,396-nucleotide deletion (chr4: 151,748,856 - 152,001,251; Figure 1C).

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 of LPS for 16 hours. Then we extracted RNA, generated cDNA and amplified a part of the LRBA gene using primers 5'GCAGAAGTCATGCTTGGACA3' and 5'TTTCGAAGTAGGGTGC AAT3'. An expected 218 bp product from the LRBA exons 19-21 was present in control, but not in patient P1 (Figure 2A). From the LPS-stimulated cells we also extracted proteins and separated them using SDS PAGE. For Western blotting we used anti-LRBA (Sigma HPA019366) and anti-actin antibodies. We found the LRBA protein with the expected mass of approximately 319 kDa in control cells, but not in cells of patient P1 (Figure 2B). We also used immunofluorescence analysis and again found that LRBA protein is expressed in the cytoplasm of cells from a healthy control, but not from patient P1 (Figure 2C)..

The LRBA gene encodes a large broadly expressed protein of unknown function that is involved in intracellular vesicle trafficking³. Recently Lopez-Herrera et al. reported five patients with different homozygous mutations in the LRBA gene that also abolished its expression⁴. 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 five patients and reduced IgM was recorded in four of those. Accordingly, all five patients have been diagnosed with childhood-onset common variable immunodeficiency (CVID). 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 may have been secondary to rituximab therapy, as has previously been described⁵. Thus, our results show that deficiency caused by mutations in the *LRBA* gene may initially present as an autoimmune syndrome without CVID. 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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Figure 1. Homozygous deletion in the LRBA gene region

A – homozygous deletion identified by ExomeDepth in the exome sequence data (estimated 99% confidence interval is shown in grey); B – validation of ~252 kb deletion using a targeted CGH array; C – sequencing of the deletion breakpoints (in patient P1 the deleted region is replaced by three nucleotides, GGT).

Figure 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; C - Immunofluorescence analysis (LRBA – green, DNA - blue, scale bars represent 10 μ m).

Table 1
Serial immunology assessments

Age (yrs)	Steroid	Rituximab	Other	ig	Infections	Lymph	CD3+ T-cells	CD4+ T-cells	CD8+ T-cells	NK cells	CD19+ B-cells	Naïve 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	1st 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	2nd 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	0.116↓	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	3rd 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 following the infectious episode. The patient received tetanus vaccination prior to first analysis as part of routine childhood vaccination. Pneumococcus vaccine was administered for the first time at the age of 9. Units for all cell counts are $\times 10^9/L$; units for IgG/A/M are g/L; units for Tet are iu/ml where >0.1 is protective. Naïve (IgD+CD27-) and class switched memory (IgD-CD27+) B-cells are shown as a % of CD19+ B-cells

Abbreviations: Yrs=years; Ig= immunoglobulin replacement; Lymph= absolute lymphocyte count; CSM= class switched memory, Tet= tetanus antibodies; PA= pneumococcus antibodies (number of positive response to 13 serotypes measured); ND= not done, Aza= azathioprine; MMF=mycophenolate mofetil; Hib= Haemophilus influenzae;

↓ or ↑ denote results below or above normal range for age respectively.

[‡] = pre vaccination;

[‡] = post vaccination with 7 valent conjugate pneumococcal vaccine;