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LETTER TO EDITOR

Novel *LRBA* Mutation and Possible Germinal Mosaicism in a Slavic Family

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To the Editor,

Lipopolysaccharide-responsive beige-like anchor protein (LRBA) deficiency was first described in 2012 [1]. Currently, the data more than 100 patients around the world has been published, presenting with a broad range of clinical, immunologic, and genetic manifestations [1–22]. This immunodeficiency can manifest as common variable immunodeficiency (CVID), autoimmune lymphoproliferative-like syndrome (ALPS)-like, immune dysregulation polyendocrinopathy enteropathy X-linked (IPEX)-like, autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED)-like, isolated diabetes mellitus, arthritis, and combined immunodeficiency (CID) with multiple autoimmune diseases [1–22].

Here, we report the clinical, genetic, and immunological data from two siblings in a Belarusian family with a homozygous, truncating *LRBA* mutation (c.2762G>C, p.Ser921Stop). Patient 1 (P1), male, was born at term to non-consanguineous parents (Fig. 1a). At his first year of life, he had several non-complicated respiratory tract infections. At the age of 4 years, he contracted infectious mononucleosis. He recovered but subsequently developed pneumonia, pancytopenia, and generalized lymphadenopathy and hepatosplenomegaly. A lymph node biopsy showed reactive changes and predominant follicular hyperplasia. Within a year, the condition progressed to autoimmune hemolytic anemia (AIHA) and hemolytic crisis which required intensive care. The patient responded to high doses of steroids, but attempts to taper medication led to relapses. He received two courses of rituximab (375 mg/m2), which stopped the hemolysis and improved the lymphadenopathy. However, multiple focal-infiltrative lesions persisted in both lungs and did not improve despite of steroids and intravenous immunoglobulin (Fig. 1b left/right).

Soon after the AIHA episode, the patient developed atopic dermatitis and diarrhea, which led to electrolytic disturbances, malabsorption syndrome, and rectal prolapse at age 9. As stool samples tested negative for common pathogens, he was diagnosed with non-specific autoimmune colitis. The combination of systemic inflammation, chronic malabsorption, and high-dose steroids stunted his growth (20.5 kg/119 cm, 9 years old) and caused Cushing syndrome, hyperparathyroidism, and secondary osteoporosis at age 8. Despite prophylactic antibiotics and immunoglobulin therapy, the patient regularly had sinusitis, ethmoiditis, and repeated pneumonias, as well as an episode of disciform keratitis.

Due to his severe disease, the patient received allogeneic stem cell transplant (HSCT) from a fully matched unrelated donor at age 10, in the absence of a definitive molecular diagnosis (Table S1). The donor cells did not engraft, and full autorecovery was evident at day 42. P1 died at 72 days post-HSCT due to systemic inflammatory response syndrome and multi-organ failure.

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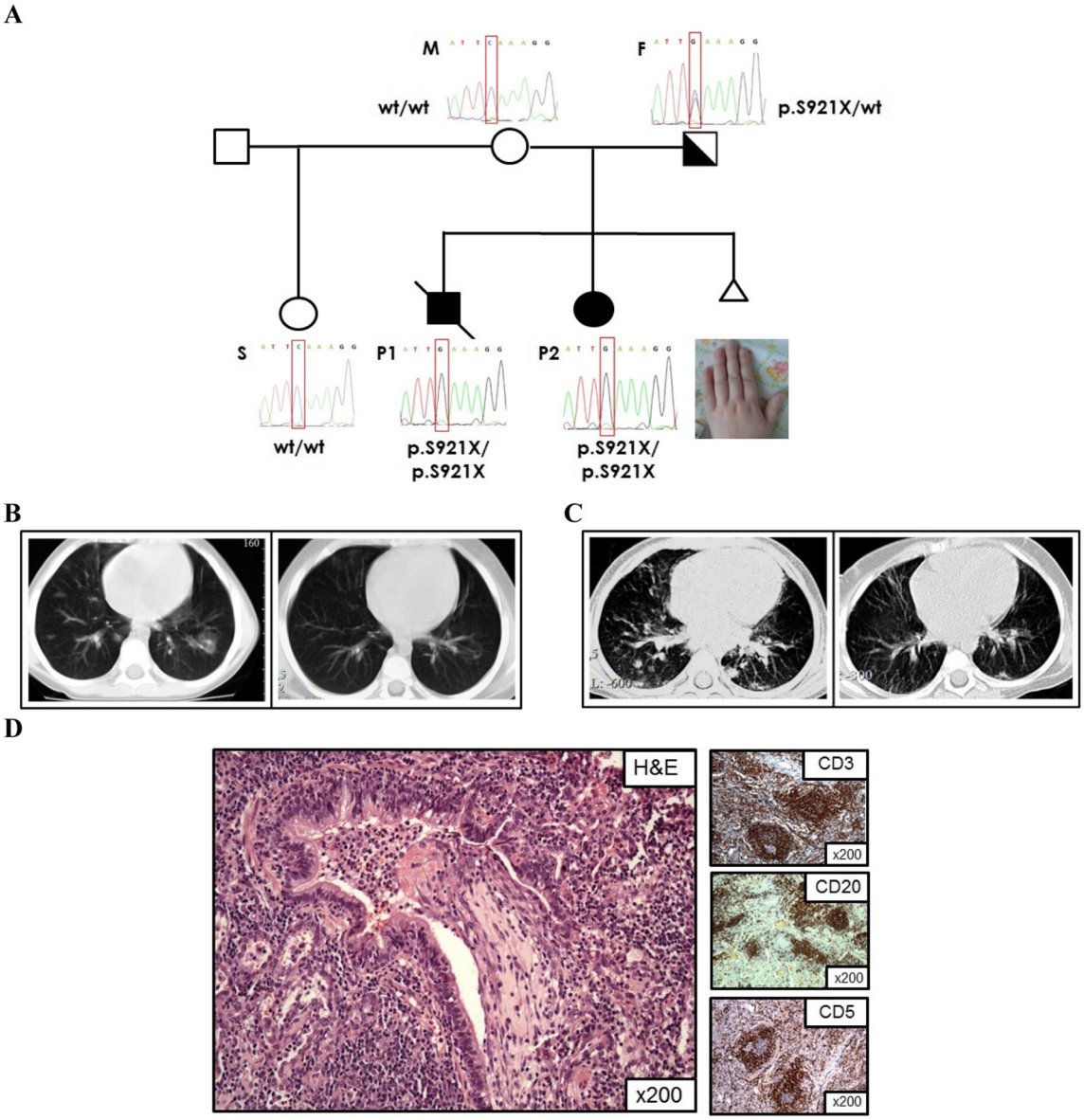

Fig. 1 Family tree and pulmonary alterations**. a** Pedigree with sequencing details**. b** (left) Chest computed tomography (CCT) of P1 at the age of 5 years revealed increased number of focal lesions, mainly in the lower lobes. **b** (right) Positive dynamics was achieved, with the reduction of major lesions and disappearance of minor ones. **c** (left) CCT of P2 (5 years old) showed generalized lymphadenopathy and multiple focal lesions up to 10 mm in both lungs. **c** (right) Improved of mean lung density due to pneumofibrotic changes and a decrease of focal lesions by 1–2 mm. **d** Lung biopsy showed interstitial pneumonia and follicular bronchiolitis. Histology of P2 lung biopsy specimens showed bronchiolitis with extensive fibrosis, and polymorphic lymphoid (CD3+ and CD20+ cells) infiltration with no granulomas was seen. Stains for bacteria, fungi, and mycobacteria were negative (H&E, Hematoxylin and eosin).

The initial basic immunologic screening (Table S2) identified a normal percentage of lymphocyte subsets and normal immunoglobulin levels for his age. Within 5 years after B cell depleting therapy, the patient had full B cell deficiency in peripheral blood and profound hypogammaglobinemia. Progression of the disease was interpreted as an adverse reaction to rituximab treatment (Table S2).

Patient 2 (P2), female, was born 2 years after the death of her brother. At age 8 months, she had a spontaneous episode of bloody diarrhea in the absence of any detectable infection. Serologic testing for celiac disease was negative, and streaks of blood in stool persisted despite gluten-free diet. Duodenal biopsy at age 5 years revealed partial villous atrophy with eosinophils, neutrophils, and inflammatory lymphocytic infiltrate in the lamina propria, as well as mild intraepithelial lymphocytic infiltration.

At the age of 2 years and 9 months, her growth stunted, and she developed lymphadenopathy, hepatosplenomegaly, and paronychia of the 3rd finger of her right hand. At age 5, she developed polyarthritis (both ankles, right knee and

interphalangeal joints) and was diagnosed with juvenile rheumatoid arthritis (RF = 14 IU/ml). Over time, a persistent cough and finger clubbing appeared. Chest computed tomography revealed extensive lymphocytic infiltration in both lungs, and she was diagnosed with granulomatous-lymphocytic interstitial lung disease (GLILD) (Fig. 1c left). She received several courses of steroids and immunoglobulin replacement therapy, which temporarily improved the situation.

Immunologic test panel at the age of 2 years 9 months revealed low levels of IgG and memory B cells (Table S2). B cell subset profiling revealed increased numbers of $CD21^{low}$ B cells and naïve B cells, and a reduction in switched memory B cells. At the age of 5, additional abnormalities were noticed in recent thymic emigrants and tregs (Table S2).

P2 underwent HSCT from a fully matched unrelated BM donor (details in Table S1). The patient is alive and well, 7 months after the transplant.

Whole exome sequencing and subsequent computational analysis of patient 1 (P1) revealed homozygous nonsense mutation in *LRBA* (Chr 4:151788827, C>G, p.Ser921Stop). Sequence data has been deposited at the European Genome-phenome Archive (EGA [23] under accession number EGAS00001002961). The mutation was validated by Sanger sequencing in both siblings. Expression of the corresponding protein product was nearly absent in P2 when measured by flow cytometry (Fig. S1). The father tested heterozygous for the observed mutation, but the mother's genomic DNA, DNA from sorted T and B, NK lymphocytes, monocytes, buccal mucosa, and fibroblasts, did not contain the mutation. Wild-type alleles were also detected in the stepsister's genomic DNA (Fig. 1a). Therefore, a heterozygous mosaic mutation (p.Ser921Stop) in mother oocytes (maternal gonadal mosaicism) was suspected to cause the sibling recurrence. Apparent homozygosity in exome sequencing can also be the result of an underlying heterozygous deletion—however, read counts were identical in all *LRBA* exomes and the nearby genomic regions and did not suggest deletion. Exome sequencing, however, cannot exclude the presence of a complex genomic rearrangement as a cause for the apparently homozygous mutation.

Germline or gonadal mosaicism, where gametes harbor genetic variants that are not present in other tissues, results from somatic mutations during the development of the reproductive organs [24]. The prevalence of this phenomenon is unknown in families with primary immunodeficiency diseases. Several conditions, in contrast, harbor de novo germline mutations, and it is possible that a proportion of the mutations are gonadal in either of the parents. As an example, in activated phosphoinositide 3-kinase (PI3K) δ syndrome (APDS I), syndrome de novo germline mutations in *PIK3CD* are present in approximately 30% of the affected families [25, 26]. The subject of gonadal mosaicism requires further study in PIDDs and other genetic diseases.

In line with previous reports [1–22], this family case demonstrates phenotypic variability between the siblings with LRBA deficiency. The first patient (P1), male, presented with severe AIHA, colitis, and bacterial infection susceptibility and died following HSCT at age 10. He did not develop hypogammaglobulinemia until after two courses of rituximab therapy. His sister did not suffer from severe bacterial infections, but developed hypogammaglobulinemia at an early age. Her autoimmune features included colitis, juvenile rheumatoid arthritis, and GLILD at the age of 5. This condition is typically a complication of CVID and mostly seen in older patients and adults [27]. The patient underwent a successful HSCT after her diagnosis, underscoring the importance of genetic studies prior transplant.

The children were born to non-consanguineous parents but with a homozygous mutation. To study the frequency of homozygosity in non-consanguineous families, we have reviewed all the published cases of LRBA deficiency, with the key findings summarized in Table S3. Among 95 (93 published + 2 Belarusian) patients described in literature, 82 (86%) of the children had homozygous abnormalities in LRBA gene. Ninety-four percent of these were born in consanguineous families, mostly from Saudi Arabia, Palestine, Turkey, Libya, Lebanon, Egypt, Morocco, Oman, Iran, and Pakistan, where marriages between relatives are part of the national culture. Homozygous mutations in non-consanguineous families were only described in two American families (patient 31, 32a, 32b). We conclude that homozygous mutations are very infrequent outside consanguineous marriages and founder populations.

In conclusion, we describe the first Belarusian family with LRBA deficiency, featuring a novel homozygous mutation in *LRBA* gene (Chr 4:151788827, C>G, p.Ser921Stop). The phenotype is consistent with the previous reports of LRBA deficiency. In our experience, definitive genetic diagnosis should be actively sought when preparing patients for HSCT. We also highlight the possibility of gonadal mosaicism in cases where the disease recurs in siblings, but one or both parents have wild-type genetic alleles.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflicts of interest.

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