Various phenotypes of LRBA gene with compound heterozygous variation: A case series report of pediatric cytopenia patients

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Abstract

Objective: LPS-responsive beige-like anchor (LRBA) deficiency is one of the most common monogenic disorders causing common variable immunodeficiency (CVID) and CVID-like disorders. However, the clinical spectrum of compound heterozygous (CHZ) LRBA variation should be extended. In this study, we presented five cases of compound heterozygous LRBA with various refractory cytopenias.

Materials and Methods: Retrospective analysis of the clinical manifestations, management, and outcomes of five cases (from five pedigrees) with LRBA gene CHZ variants which initially manifested as single/multilineage immune cytopenias was performed.

Results: 1. Gene variations: All five patients inherited the compound heterozygous LRBA variations from their parents which were thought to be pathogenic. BEACH, DUF4704, and LamG were the main affected domains of LRBA gene in this case series. 2. Immune dysregulation of clinic: (1) Hypogammaglobulinemia were recorded in four patients, and the proportion of Treg was decreased in two patients. Only one patient had been with increased TCRαβ+CD4/CD8 double-negative T cells (DNT). (2) Lymphoproliferative manifestations were seen in three patients. (3) All five patients were complains with cytopenia, although they showed different clinical manifestations. None of the parents was asymptomatic. (4) Other immune disorders: P5 also had relapsed infections and autoimmune endocrinopathy. 3. Management and outcomes: P1 and P5 responded well to immunomodulatory therapy and P3 was effectively treated with hemophagocytic lymphohistiocytosis (HLH) first-line regimen chemotherapy. P4 showed no responses to steroids and IVIG. However, TPO-R agonist was effective.

Conclusion: Unlike homozygous mutations, compound heterozygous LRBA variation should always be kept in mind for the various phenotypes and different treatment responses.

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Keywords
LPS-responsive beige-like anchor deficiency, common variable immunodeficiency, autoimmune lymphoproliferative syndrome, hypogammaglobulinemia, immunomodulatory therapy

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What is known–what is new

What is known

- LRBA defects should always be kept in mind as a differential diagnosis for patients with autoimmune disease affecting multiple organs, chronic diarrhea, and organomegalies.
- In patients refractory to corticosteroids, treatment with immunosuppressive drugs such as azathioprine, 6-mercaptopurine, tacrolimus, mycophenolate mofetil, infliximab, and sirolimus have been reported.

What is new

- Various phenotypes of pediatric patients with compound heterozygous LRBA variation on set as cytopenia are summarized.
- Compound heterozygous LRBA variation has different clinical manifestation which needs personalized treatment.

Introduction

LPS-responsive beige-like anchor (LRBA) deficiency is a primary immunodeficiency (PID) categorized as common variable immunodeficiency associated with autoimmune manifestations and inflammatory diseases. According to the recent reports, LRBA deficiency is one of the most common monogenic disorders causing CVID and CVID-like disorders. A decrease in LRBA protein function leads to the diminished expression of cytotoxic T-lymphocyte-associated protein-4 (CTLA-4) on the surface of Treg cells, in particular patients with phenotypes resembling autoimmune lymphoproliferative syndrome (ALPS), including autoimmune cytopenias, and lymphoproliferation. However, the clinical spectrum of compound heterozygous LRBA variation should be extended. In this study, we presented five compound heterozygous LRBA patients with refractory cytopenias. Unlike homozygous mutations, there were differences in clinical manifestations of compound heterozygous LRBA variation. The aim of this study was to explore and compare the LRBA-related cytopenia in five different compound heterozygous LRBA patients. We also analyzed their clinical and laboratory features to extend the clinical spectrum of compound heterozygous LRBA variation.

Materials and methods

Patients and immunological data collection

This case series study was conducted from December 2017 to February 2019. A total of five LRBA gene CHZ variation patients at the hematology and oncology departments in our hospital were enrolled into this study. Clinical and immunological laboratory characteristics of the patients are described in Tables 1 and 2. LRBA deficiency or knockdown may increase CTLA4 turnover, which resulted in reduced levels of CTLA4 protein in FoxP3+ regulatory and activated conventional T cells. We also detected the LRBA and CTLA4 expression by flow cytometry.

Among five patients, the elevated proportion of P1 (>1.5% of total lymphocytes or >2.5% of CD3+ 87 lymphocytes) of DNTs/CD3+ (3.41%) and chronic (>6 months), nonmalignant, noninfectious splenomegaly made us consider the diagnosis of required criteria of ALPS; however, no FAS, FASL, and Caspase 10 mutations were found. The proportion of regulatory T lymphocyte (Treg) was decreased, while the expression of CTLA-4 was not detected by flow cytometry. Not only the cytopenia and lymphoproliferative, P1 was found to have lung infiltrates as well. His chest CT showed multiple nodular lesions with different size and small plaque grinding glass lesions. After treatment with sirolimus (1.5 mg/m2, aimed blood concentration 9–12 ng/L), subsequently, the patient’s platelets and hemoglobin improved rapidly and lung CT was improved (Figure 1).

The main clinical characteristic in P2 was pancytopenia without autoimmune disease, who developed a bone marrow failure disease in the future. The expression of LRBA was normal by flow cytometry in P2 (Figure 2).

P3 was initially diagnosed with HLH due to fever, splenomegaly, hemopenia, low fibrinogen, high TG, elevated ferritin, and hemophagocytosis in bone marrow.

P4 and P5 were diagnosed with CVID. The clinical characteristics in P4 were autoimmune thrombocytopenia, hypogammaglobulinemia, and lower CTLA4 expression by flow cytometry (Figure 3). P5 also had intermittent diarrhea and type I diabetes mellitus that lasted over 2 years. His thyroid function showed the decreased T3 and T4 serum level, and he was diagnosed with autoimmune thyroiditis. The expression of LRBA was also normal by flow cytometry in P5.
Gene defects in patients
All patients underwent Sanger sequencing after NGS-based gene panel screening. Genomic DNA was extracted from bone marrow using the QIampDNA Blood Kit (Qiagen) according to the manufacturer’s instructions. Library Expansion PCR and Product Purification: PCR reactions were carried out in H2O 40 μL, Barcode 1 μL, PE 1.01 μL, PCR Reaction Buffer 27 μL, and PCR Enzyme 1 μL. PCR conditions were as follows: a denaturation step at 98°C for 2 min; 8 cycles at 98°C for 30s, 65°C for 30s, and 72°C for 30s; and a final extension step at 72°C for 5 min. The product of PCR was purified by magnetic beads. The ratio of magnetic beads to samples was 1.5:1.

Table 1. Patients’ clinical characteristics.

|       | P1     | P2     | P3     | P4     | P5     |
|-------|--------|--------|--------|--------|--------|
| Age (year) | 4      | 2      | 13     | 4      | 3      |
| Sex    | M      | M      | M      | F      | M      |
| Follow-up time (month) | 19     | 22     | 37     | 28     | 21     |
| Hemoglobin (g/L) | 85     | 77     | 109    | 73     | 88     |
| Leukocytes (×10^9/L) | 1.48   | 2.79   | 1.69   | 3.4    | 4.46   |
| Platelets (×10^9/L) | 16     | 25     | 58     | 1      | 0      |
| Lymphoproliferation | Splenomegaly and enlarged lymph nodes | Hepatosplenomegaly | N      | Enlarged lymph nodes |
| Infection events | Pneumonia | N      | Fever and oral ulcer | N      | Respiratory and digestive tract infection |

Other autoimmune diseases

Diagnosis | ALPS | AA\(^2\) | HLH | CVID | CVID |
|----------|------|----------|-----|------|------|
| Treatment and dose | Sirolimus: 1.5 mg/m^2 (blood concentration range: 4.27–10.3 ng/mL) | CsA: 15 mg/kg/d (blood concentration range: 100–150 ng/mL) | BCH-HLH-2004 regimen chemotherapy (dexamethasone, CsA, and VP-16) | IVIG: 800 mg/kg HDD: 0.6 mg/kg/d, 4 days | Eltrombopag: 25 mg/d |

HDD: High-dose dexamethasone; CsA: cyclosporin A; IVIG: intravenous immunoglobulin; ALPS: autoimmune lymphoproliferative syndrome; AA: aplastic anemia; HLH: hemophagocytic lymphohistiocytosis; CVID: common variable immunodeficiency disease.

Table 2. Patients’ immunophenotypic analysis results.

|       | P1     | P2     | P3     | P4     | P5     |
|-------|--------|--------|--------|--------|--------|
| Absolute value of lymphocytes (/μl) | 3348   | 4465   | 1770   | 2793   | 2430   |
| Total T lymphocytes absolute value (CD3+, CD19−) (/μl) | 2980   | 2893   | 1327   | 1494   | 1348   |
| Total B lymphocytes absolute (CD3−, CD19+) value (/μl) | 164    | 1192   | 242    | 1221   | 959    |
| Helper T lymphocytes (CD3+, CD4+) % | 37.6   | 35.7   | 27.9   | 20.2   | 44.4   |
| Inhibitory T lymphocytes (CD3−, CD8+) % | 42.2   | 22.1   | 42.4   | 31.6   | 17.8   |
| CD4/CD8 | 0.89   | 1.62   | 0.66   | 0.64   | 2.5    |
| Regulator T lymphocytes CD3+CD4+CD25+FOXP3+ (%) | 1.81 (low) | 9.17   | 4.32   | 1.78 (low) | 5.13   |
| CD3+TCRαβ+CD4-CD8-DNT% | Positive | N | N | N | N |
| IgA (g/L) | Low    | Low    | Low    | Low    | N      |
| IgG (g/L) | Low    | N      | N      | N      | N      |
| IgM (g/L) | Low    | Low    | Low    | Low    | N      |
| Coombs test | IgG +++ | N | N | N | IgG ++ |

The Coombs test had not been detected in P3.
Figure 1. Comparison of lung CT before and after therapy: (a) Before and (b) after treatment.

Figure 2. LRBA expression of peripheral blood (left) and bone marrow (right) in P2.

Figure 3. CTLA4+ expression in normal people (left) and P4 (right).
Agarose gel electrophoresis was used for qualitative examination of the products of PCR. Agarose gel electrophoresis quality detection standard implied that if a clear and slightly dispersed band around 200–500bp was obtained, the database could be successfully built. After bioinformatics analysis and variant selection, possible pathogenicity mutations were analyzed based on American College of Medical Genetics and Genomics (ACMG) recommendation and clinical features of patients.

**Statistical analysis**

Statistical analysis of the acquired data was performed using GraphPad Prism 7.01 software (GraphPad Software Inc., CA, USA). All probability values were two-tailed, and a \( p \) value < 0.05 indicated a statistically significant difference.

**Results**

All patients underwent Sanger sequencing by NGS-based gene panel screening including 4 cases used whole exome sequencing and 1 case had hematological and immunological disease gene panel (supplementary file). Compound heterozygous variations were found in all patients. It was confirmed that each of the distinct variations was from their parents. The specific generation sequencing results are shown in Table 3 and gene sequencing map of P1–P5 is shown in Figure 4.

**Genotype**

There was no other cytopenia-related pathogenic gene variation detected in all these five patients, so the LRBA CHZ variations were considered to be the pathogenic variations. The variation of c.928C > T in P1 was point LamG super family (see Table 3 and Figure 5), which is a functional unknown domain (DUF4704). The two variations were preliminarily identified as pathogenic mutations, for which the pathogenic criterion was weighted as PS1 (strong).

The c.7049G > T found in P2, which was a missense variation, involves the BEACH (Beige and Chediak-Higashi) domains of membrane transport. This domain exists in the conserved protein family in the whole eukaryotes. The other c.5003dupC was frameshift mutation which was preliminarily determined to be pathogenic mutation. The two variations were preliminarily identified as pathogenic mutations, for which the pathogenic criterion was weighted as PP4 (very strong).

In P3, both c.7049G > T and c.7382C > T were all missense mutations and the variation sites also involved the BEACH domains. The variation of c.7382C > T does not belong to the polymorphism site and it is rare in the population, for which the pathogenic criterion was weighted as PM2 (moderate). The variation of c.7049G > T was weighted as PP4 (supporting evidence). There was no literature report at present for these two variations.

In P4, c.1570G > A mutation was missense mutation and the pathogenic criterion was weighted as PS1 (strong). The c.1570G > A mutation is a functional unknown domain (DUF4704), which exists in the eukaryotes of nerve cell proteins. The other c.6047–9A > G was found, located in -9 position which might result in amino acid change from splicing mostly and the pathogenic criterion was weighted as PM2 (moderate).

In P5, the variation of c.7092_7093delTT (deletion) was frameshift mutation, which does not belong to the polymorphism site and occurs very low in the population. The mutation site is also the BEACH domain. There was another c.1549G > T, which was nonsense mutation and also did not belong to the polymorphism site. The mutation is a domain with unknown function (DUF4704). The two variations were preliminarily identified as pathogenic variations, and they were weighted as PVS1 (very strong) based on pathogenic criterion.

**Immunomodulatory therapies and outcome**

Steroids, mycophenolate mofetil, and IVIG (800 mg/kg) were common immune suppressive therapies used in relapsed and refractory autoimmune cytopenia.

After 3 months therapy with sirolimus, the blood routine platelets increased to normal, the proportion of Treg also increased, and splenomegaly and lung CT absorption was improved in P1.

P2 was considered as an aplastic anemia phenotype with ineffectiveness of blood transfusion. The patient was treated with cyclosporine A in the outpatient.

P3 was diagnosed with HLH and was treated with BCH-HLH-2004 regimen chemotherapy (including dexamethasone, CsA, and VP-16) for 8 weeks. Fever and blood routine examination returned to normal level after 1 month treatment. IgA and IgM were still low after six months, and there was no recurrence of hemophagocytosis-related indexes.

At the initial stage of the disease, human immune globulin and steroids were effective. However, the platelet, which was repeatedly monitored, decreased in P4. Intermittent administration of high doses of dexamethasone combined with immune globulin, rituximab, and sirolimus
Table 3. Detailed LRBA genetic results in patients.

| Patients | P1 | P2 | P3 | P4 | P5 |
|----------|----|----|----|----|----|
| cDNA mutation | c.5003dupC | c.928C>T | c.7049G>T | c.52G>A | c.7382C>T |
| Protein level mutation | p.S1669VfsX18 | p.R310X | p.S23501 | p.G18R | p.P246IL |
| Mutation mode | Frameshift | Nonsense | Missense | Missense | Missense |
| Affected exon | exon30 | exon18 | exon47 | exon2 | exon49 |
| Affected domain | LamG | BEACH | - | BEACH | BEACH |
| PolyPhen2 | NA | NA | 0.534 | 0.102 | 0.269 |
| SIFT | NA | NA | 0.003 | 0.011 | 0.026 |
| CADD | NA | 37 | 29 | 23.2 | 25.5 |
| MAF | NA | NA | 0.0113 | 0.0035 | 0.0001 |
| gnomAD | NA | 0.000004077 | 0.0009 | 0.0017 | 1.221e-05 |
| Popmax frequencies | - | 0.000003994 | 0.0114 | 0.0035 | 0.0002 |

*a* The frameshift mutation is identified as pathogenic variation, although it is predicted that it does not affect any domains by software.

*b* The variation is preliminarily determined to be of unknown clinical significance and does not affect any domains by software.

*c* The variation is located in intron which does not affect any domains.

*d* “-” means no data of variation frequencies in the normal person in database currently.
Figure 4. Gene sequencing map of P1–P5.
Figure 4. Continued.
were all ineffective. Finally, TPO-R agonist (oral eltrombopag) was effective. The platelet increased to normal in P5 after 4 days of immunotherapy with immune globulin and dexamethasone. P5 relapsed with platelet dropped again 2 years later and was effective with dexamethasone treatment as well.

None of the five patients developed malignancy during or after immunotherapy.

Discussion

LRBA deficiency was first described in 2012 as an autosomal recessive disorder caused by biallelic mutations in the LRBA gene (OMIM #614700). Reduced T regulatory (T reg) cells ratio, low CTLA4 and Helios could be detected in LRBA deficiency. However, increased B-cell apoptosis, low levels of IgG+/IgA+CD27+switched-memory B cells, reduced B proliferative capacity, and impaired activation (using CD138 staining), which were the immunopathological mechanisms identified had in PID patients. It may also be accompanied by various autoimmune diseases, hypogammaglobulinemia, and recurrent infection, including idiopathic thrombocytopenic purpura, autoimmune hemolytic anemia, and inflammatory bowel disease. However, further reports described LRBA deficiency as a clinically variable syndrome with a wide spectrum of clinical manifestations. The literature also showed that individuals of homozygous LRBA mutations had hypogammaglobulinemia and autoimmunity, whereas heterozygous individuals were healthy. These mutations were absent in healthy controls. A systematic review study evaluated 109 patients (52 males, 47 females, and 10 with unknown gender) with the molecular diagnosis of LRBA deficiency. Various autoimmune conditions, including hematological, gastrointestinal, endocrine, neurological, and rheumatologic disorders, were reported in 84 patients (82%). Autoimmune hematologic disorders including autoimmune hemolytic anemia and immune thrombocytopenic purpura were the most frequent autoimmune complications (50% and 48%, respectively). In our study, all patients had cytopenias, including refractory and relapsed immune cytopenia. But not all patients showed hypogammaglobulinemia and their diagnoses were different. P1 was diagnosed with ALPS, P2 had bone marrow failure disease, and P3 had HLH, while P4 (CVID) had refractory thrombocytopenia and P5 (CVID) also had autoimmune polyglandular disease and recurrent infections. I wonder if it can be explained that their different clinical manifestations are due to the compound heterozygous variations and different variation locations.

The LRBA gene is located on 4q31.3, contains 57 exons, and encodes a protein containing 2851 amino acid residues, which belongs to the enigmatic class of BEACH domain–containing proteins. These mutations are distributed throughout the gene and essentially include missense mutations, splice site mutations, small indels, nonsense mutations, and large structural rearrangements. Our five patients had compound heterozygous LRBA variations from their parents. But there was no clinical manifestation of immune dysregulation to their parents. Among several domains (drawn by the NCBI databases), BEACH, DUF4704, and LamG were the main affected domains in this cohort.

The BEACH domain has attributed various cellular functions, typically involving intracellular protein, membrane transport processes, and aberrant autophagy. The variable symptoms include autoimmunity, chronic diarrhea, B-cell deficiency, and hypogammaglobulinemia. P3 who initially diagnosed as HLH had coagulation dysfunction, hypogammaglobulinemia, and aberrant autophagy. Single variation of BEACH occurred in P2 and P5 with different manifestations. The symptoms include autoimmunity, chronic diarrhea, B-cell deficiency, and hypogammaglobulinemia all occurred in P5, but P2 only had...
hypogammaglobulinemia. Laminin G (LamG) domain is a signal transduction through steroid receptors on the cell surface and also can be binding sites of some cytokine-mediated adhesion, migration, and differentiation of cell adhesion molecules. However, the relation with the immunity dysfunction was not clear. Single variation of LamG occurred in P1, which was a nonsense mutation. In addition, the function of the DUF4704 and other domain still remains unclear. We could not confirm the different clinical manifestations should be all related to the variation site in compound heterozygous variation and wondered if the same genetic changes were consistent with same symptoms because the patients with the same variation combinations were not found in our study. The precise role that LRBA plays in the pathogenesis of these disorders needs to be investigated further.

LRBA is highly expressed in immune cells such as T and B cells. Currently, there are some clinical reports about patients harboring biallelic mutations in the LRBA gene and CTLA4 (CD152). More than 70% of the LRBA-deficient patients have reduced levels of Treg, which may be related to the low surface expression of CTLA4. There were only two patients with decreased Treg. The proportion of Treg was decreased in P1 (ALPS) and P4 (CVID), which were all with lower expression of CTLA4. But the further confirmation was needed to do in other patients whether that the reduced levels of Tregs related with low surface expression of CTLA4.

LRBA has a pivotal role in the intracellular trafficking of cytotoxic T-lymphocyte protein-4. In the current study found a lower level of CTLA4 expression in LRBA-deficiency patients. The expression of CTLA4 was reduced in P1 and P4. And the expression of LRBA was normal in P2 and P5, especially in P5 with pathogenic LRBA gene mutations. So it could be supposed that if the function of LRBA was abnormal consistently, the function of LRBA proteins needs to be further confirmed in patients with LRBA gene CHZ variations.

Immunologic abnormalities reported in homozygous LRBA mutation patients also include deficient B cells and decreased IgG antibody production. But in this study, only P1 had decreased IgG and B cells. Progressive decrease in IgA and IgM levels was seen in four patients (P1–P4), except in P5. Patients with decreased IgA and IgM instead of IgG antibody production was one of the features of the immunological changes in compound heterozygous variations in our study. The proportion of B cells and Tregs, immunoglobulins, and CTLA-4 expression were all decreased in P1, and these indicators were gradually restored after immunotherapy during the follow-up.

LRBA deficiency is a recently defined defect, with variable presentations in different patients; a single, definitive treatment option is thus not yet available. To date, different agents have been applied in the treatment of LRBA deficiency. Some patients also benefit from hematopoietic stem cell transplantation (HSCT). In a retrospective study of 76 patients with LRBA deficiency from 29 centers (median follow-up, 10 years; range, 1–52), 24 underwent HSCT from 2005 to 2019. The overall survival rate after HSCT (median follow-up, 20 months) was 70.8% (17 of 24 patients); all deaths were due to nonspecific, early, transplant-related mortality. Currently, 82.7% of patients who did not receive a transplant (43 of 52; age range, 3–69 years) are alive. Of 17 HSCT survivors, seven are in complete remission and five are in good partial remission without treatment (together, 12 of 17 [70.6%]). In contrast, only 5 of 43 patients who did not receive a transplant (11.6%) are without immunosuppression. Due to different clinical manifestations, we used different therapies.

Administration of sirolimus was effective in P1 (ALPS) because of abnormal DNT/Treg axis which can be re-balanced by mTOR signal pathway inhibitor sirolimus. Carrying immunodeficiency gene, P3 was diagnosed with HLH without DNT cell amplification and was effectively treated with HLH first-line regimen chemotherapy. Oppositely, there was report about homozygous missense variation on the UNC13D gene, which might result in familial HLH, leading to ALPS-like disease. There were many similarities in the clinical manifestations of HLH and ALPS, but the pathogenesis, prognosis, and treatment were all different. Therefore, DNT cell amplification is only present in ALPS and is a reflection of the differences in ALPS immunopathology.

Different from other patients, there was no autoimmunity and the whole blood cells decreased gradually in P2. The treatment was initiated with CsA for bone marrow failure disease, and the curative effect remains to be observed.

The common treatment of CVID with cytopenia was immunosuppressive drugs, including corticosteroids, intravenous immunoglobulin, and rituximab therapy. Most patients have a therapeutic response to immunosuppressive drugs. Two CVID patients in our study (P4 and P5) had complete response to corticosteroids and immunoglobulin initially. However, P4 and P5 relapsed. Intermittent administration of dexamethasone combined with immunoglobulin, rituximab, and sirolimus were all ineffective for P4. Because of the failure of immunosuppressive treatments of P4, we tried TPO-R agonist eltrombopag in P4 and it was effective in the end. More recently, studies have suggested the abatacept, a CTLA4-immunoglobulin fusion protein for controlling disease-related immune dysregulatory phenotypes. It can be used for the refractory immune cytopenia patients with decreased CTLA4 expression.

Therefore, a summary of these five cytopenia (including refractory and relapsed) patients showed that not only the clinical manifestations and treatments were different, but
the therapy responses as well. However, it is not yet possible to draw regular conclusions because of the limitation of the scale of our patients. There are limitations in this study; the calculation and justification of the sample size could not be done because of the sample size limitation of these cases. The relation of the genotype and phenotype in the LRBA gene with compound heterozygous variation may be figured out in the future by the enlargement of scale of this group of patients.

Conclusion

Unlike homozygous mutations, compound heterozygous LRBA variation should always be kept in mind for the various phenotypes and different treatment responses.

Authors’ Contributions

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Jiafeng Yao, Hao Gu, and Wenjun Mou; Zhenping Chen, Jie Ma, Honghao Ma, Nan Li, Rui Zhang, and Jin Jiang recruited all the subjects and collected the written informed consent. The first draft of the manuscript was written by Jiafeng Yao and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Availability of data and material

The data that support the findings of this study are available on request from the corresponding author. Additional declarations for articles in life science journals that report the results of studies involving humans and/or animals. Patients (or their parent or legal guardian in the case of children under 16) signed informed consent regarding publishing their data and photographs.

Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Capital Medical University. Approved No. of ethic committee: IEC-C-008-A08-V.05.1.

Informed consent

Written informed consent was obtained from the legally authorized representative of the minor subject for the publication of the case series.

Consent to participate

We invite you to participate in the establishment and application of a standardized diagnosis and treatment system for childhood immune thrombocytopenia approved by the Capital Medical University. This study complies with the Declaration of Helsinki and the principles of clinical trial quality management practices and has passed the review of the ethics committee of Beijing Children’s Hospital affiliated to Capital Medical University. Before deciding whether to participate in this study, please read the following as carefully as possible for you and your parents. The clinicians could help you understand the research and why you want to conduct the research, the procedures and duration of the research, the benefits, risks and discomforts that may be brought to you after participating in the research, you may not fully understand the content described in this consent form Where, please consult the researcher or doctor for a detailed explanation. If you wish, you can also discuss with your relatives and friends to help you make a decision.

Consent for publication

All authors have read and approved the final manuscript. All parents signed informed consent forms and approved the final manuscript.

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Supplemental Material

Supplemental material for this article is available online.

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### Appendix

**Abbreviation**

| Abbreviation | Description |
|--------------|-------------|
| AA           | aplastic anemia |
| ALPS         | autoimmune lymphoproliferative syndrome |
| ACMG         | American College of Medical Genetics and Genomics |
| CHZ          | compound heterozygous |
| CVID         | common variable immunodeficiency |
| HLH          | hemophagocytic syndrome |
| LRBA         | LPS-responsive beige-like anchor |
| NGS          | next-generation panel screening |
| PID          | primary immunodeficiency |