LIG4 Syndrome: Clinical and Molecular Characterization in a Chinese Cohort

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Research

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Abstract

Background: DNA Ligase IV (LIG4) syndrome is a rare disease with few reports to date. Patients suffer from a broad spectrum of clinical features, including microcephaly, growth retardation, developmental delay, dysmorphic facial features, combined immunodeficiency, and malignancy predisposition. There is thought to be a potential association between genotypes and phenotypes. Here, we investigated the characteristics of LIG4 syndrome in a Chinese cohort.

Results: All seven patients had growth restriction. Most patients (6/7) had microcephaly (< -3 SD). Recurrent bacterial infections of the lungs and intestines were the most common symptoms. One patient had myelodysplastic syndromes. One patient presented with an inflammatory bowel disease (IBD)-like phenotype. Patients presented with combined immunodeficiency. The proportions of naive CD4+ and naive CD8+ T cells decreased notably in five patients. All patients harbored compound heterozygous mutations in the *LIG4* gene, consisting of a missense mutation (c.833G>T, p.R278L) and a deletion shift mutation, primarily c.1271-1275delAAAGA (p.K424Rfs). Two other deletion mutations, c.1144-1145delCT and c.1277-1278delAA, were novel. Patients with p.K424RfsX20/p.R278 might lead to milder dysmorphism, but more significant IgA/ IgM deficiency, compared with the frequent genotype p.R814X/p.K424RfsX20 reported. One patient underwent umbilical cord blood stem cell transplantation (UCBSCT) but died.

Conclusions: This study reported the clinical and molecular characteristics of a Chinese cohort, further expanding the phenotypic and genotypic spectrum, and the understanding of genotype-to-phenotype correlations in LIG4 syndrome. The results may help to characterize the status of the disease in China.

Background

DNA ligase IV (LIG4) syndrome is an exceptionally rare autosomal recessive disorder that belongs to the group of hereditary diseases associated with impaired DNA damage response mechanisms. The distinct features of LIG4 syndrome patients are microcephaly, growth retardation, developmental delay, dysmorphic facial features, variable immunodeficiency, pancytopenia, malignancy predisposition and pronounced clinical and cellular radiosensitivity [1].

The DNA ligase IV is one of the proteins that functions in the nonhomologous end-joining (NHEJ) pathway, a major mechanism involved in the repair of DNA double strand breaks (DSBs) in mammals [2], leading to increased sensitivity to ionizing radiation. DSBs are also involved in the processes of class switch and V(D)J recombination during immune development; thus, the failure to carry out the processes caused by NHEJ deficiency confers (severe) combined immunodeficiency.

The *LIG4* gene, mapping to chromosome 13q33-q34, has a complex structure formed by four domains: the DNA-binding domain (DBD), adenylation domain (AdD), oligo-binding domain (OBD) and XRCC4-binding domain (XBD). LIG4 syndrome is caused by homozygous or compound heterozygous mutations in the *LIG4* gene, the most common genotype of which is considered as p.R814X/p.K424RfsX20 [3]. Moreover, Murray et al.[4] first proposed a genotype–phenotype correlation between the position of truncating mutations associated with p.R814X and disease severity.

Although this disease was first described nearly 30 years ago, only a few cases have been reported to date [3-26]. In this study, we reported the clinical, immunological and genetic characteristics of 7 Chinese patients with LIG4 syndrome.

Methods

This study was approved by the Ethics Committee of the Children's Hospital of Fudan University. Informed consent was obtained from the parents of the patients.

1. **Patients and Clinical Data**

LIG4 syndrome was suspected based on clinical manifestations of patients referred to our hospital between July 2014 and December 2019, which was further confirmed by immune function and gene detection in this study. The relevant data were summarized in detail. Previous patients and mutations reported in PubMed Medline (https://www.ncbi.nlm.nih.gov/pubmed/) were reviewed and compared.

2. **Immunological Function**

The immunoglobulins, including IgG, IgA, and IgM, were detected by nephelometry, while the analyses of lymphocyte subsets were performed on a FACSCalibur flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA) and FACSDiva software (BD Biosciences). Briefly, samples were processed using the standard 'stain – lyse – wash' technique. The peripheral blood mononuclear cells (PBMCs) were stained for surface antigens with a panel of monoclonal antibodies and isotype control antibodies followed by red blood cell lysis (BD FACS) and washing with PBS. The following validated antibodies were used for flow cytometry: anti-CD3 (UCHT1), anti-CD8 (RPAT8), anti-CD27 (M-T271), anti-CD45RA (HI100), anti-CD4 (RPA-T4), anti-TCRαβ (T10B9.1A-31), anti-TCRγδ (B1), anti-CD19 (HIB19), anti-CD24 (ML5), anti-CD38 (HIT2), and anti-IgD (IA6-2) (all from BD Biosciences).

3. **Molecular Analysis**

Genomic DNA was extracted from the peripheral blood of the patients and their parents using the QIAamp DNA Blood Mini kit (Qiagen, Hilden, Germany). DNA quality was assessed using a NanoDrop ultraviolet spectrophotometer (Thermo Fisher Scientific, USA).

Next-generation sequencing was performed using a panel that included all previously reported immunodeficiency genes [27]. Genomic DNA fragments of patients were ligated with adaptors such that two paired-end DNA libraries with insert sizes of 500 bp were formed for all samples. The DNA libraries after enrichment were sequenced on the HiSeq 2000 platform in accordance with the manufacturer's instructions (Illumina, San Diego, CA). The variants were annotated by ANNOVAR and VEP software and predicted with SIFT, PolyPhen-2 and MutationTaster. The mutations were confirmed by Sanger sequencing.

Results

1. **Clinical Manifestations**

Overview

Seven patients (4 males and 3 females) were diagnosed over a 5-year period in our center. The average age of morbidity was 5.3 months (range, 1 week-14 months), while the mean time of diagnosis was extended to 18.4 months. All of these cases were full-term infants. No patients were born out of consanguineous marriages. No disease-related family histories were found, except that the mother of patient 2 (P2) and P5 had previous pregnancy with embryo growth arrest. The clinical findings are summarized in Table 1.

Microcephaly and growth restriction was obvious in these patients. The head circumference of six patients was more than 3SD below the population mean of the same age and same gender. Moreover, almost all the patients had postnatal underweight (6 of 7 < -3SD) and stature lower than standard (2 of 7 < -3SD) such that the weight loss was more pronounced (Fig. 1). Three full-term, small-for-gestational-age infants indicated intrauterine growth retardation during the early life stage. Two patients had clinically developmental retardation, which was mainly manifested as delayed language and motor retardation compared with healthy children of the same age. Head MRI of five patients showed no abnormalities. Intelligence assessment of scales or questionnaires was just completed in P1 and P2. However, the typical facial deformity was not found in our study.

Infection Characteristics

The patients were at high risk for respiratory and intestinal infections. One patient presented with omphalitis shortly after birth. Then, in a few months, these patients developed such conditions as pneumonia, diarrhea, thrush, and otitis media. *Acinetobacter, Enterobacter cloacae, Flavobacterium menigosepticum* and *Haemophilus influenzae* were usually detected from the secretion of lower respiratory tracts by bacterial culture. *Salmonella* was cultured in the stool of two patients with diarrhea. *Parainfluenza virus* was also detected repeatedly from sputum in P1 and P4. Despite oral antiviral and interferon inhalation therapy, the virus was difficult to clear. Pulmonary CT of P1 showed diffuse interstitial changes (Fig. 2), which were considered to be highly correlated with viral infection. Moreover, metagenomic sequencing of the bronchoalveolar lavage fluid sample in P4 detected *Pneumocystis jiroveci* (reads, 189), partly causing extensive lung involvement.

Autoimmune and Other Characteristics

Patients might also present as noninfectious manifestations. Three patients developed thrombocytopenia, which could increase after intravenous immune globulin (IVIG) infusion. This finding suggested potential autoimmune factors, although the result of this routine autoantibody test was negative. It is worth noting that P2 was initially admitted to our hospital due to oral ulcers and intermittent fever. The anti-infection treatment with multiple antibiotics, such as mepin, vancomycin, SMZ and itraconazole, was ineffective, while only respiratory syncytial virus was positive in etiology. Colonoscopy revealed multiple ulcers (Fig. 2), and no etiology was found in biopsy samples by metagenomic sequencing. After steroid administration, body temperature was lower than before, and oral ulcers were slightly better.

Other blood system abnormalities, such as leukopenia, occurred in two children. A laboratory examination of P6 suggested pancytopenia at 26 months; then, she was suspected of having “myelodysplastic syndromes” based on a bone marrow aspiration smear, but a bone marrow biopsy was not confirmed further. Three cases had skin lesions, one of whom (P4) presented with erythroderma and one with hypopigmentation. P5 primarily suffered from recurrent eczema and skin peeling, accompanied by increase of eosinophilic cell count to 2.7-8.1×10^9/L.

2. **Immune Function Evaluation**

Flow cytometric analysis of peripheral blood usually showed a significant decrease in the absolute numbers of CD19+ B cells and CD3+ T cells (especially CD4+ T cells) but normal or decreased numbers of NK cells (Table 2). However, the count of lymphocytes was decreased slightly in P5, whose chimerism test was not performed to exclude maternal-fetal cellular trafficking. Peripheral blood lymphocyte subsets of five patients (P1, P2, P3, P6, and P7) were analyzed in detail. All of these cases were significantly decreased in naive Th cells (CD4+CD45RA+CD27+) and naive cytotoxic T cells (CD8+CD45RA+CD27+) compared to the reference values in healthy children in China [28]. The IgG levels of P5 and P7 were significantly lower than the normal reference value accompanied by reduced IgM. Other patients had normal levels of IgG before or after IVIG therapy.

3. **Genetic Characteristics**

Mutations in the *LIG4* gene were identified in all 7 cases. Patients harbored compound heterozygous mutations consisting of a missense mutation and a deletion shift mutation. The common missense mutation c.833G>T in exon 2, which caused an amino acid change of Arg to Leu, was identified. In addition, c.1271-1275delAAAGA was found in four patients and became the most frequent deletion mutation. Three other deletion mutations were also detected, specifically c.1144-1145delCT, c.1277-1278delAA and c.1270-1274delAAAAG (Table 3). Mutations c.1144-1145delCT and c.1277-1278delAA had not been reported previously, and predicted to be deleterious.

The most frequent genotype in our cohort was p.R278L/p.K424RfsX20 and found in five children. Further comparison with the genotype p.R814X/p.K424RfsX20 reported in previous literatures (Additional file 1: Table S1), we discovered a little difference in phenotype. Microcephaly and facial dysmorphism were more common in patients with genotype p. R814X/p.K424RfsX20 than genotype p.R278/p.K424RfsX20. Patients with p.R814X/p.K424RfsX20 had mainly IgG deficiencies, while patients with p.R278/p.K424RfsX20 were accompanied by a decrease in IgA and IgM levels.

4. **Treatment and Outcome**

The patients underwent anti-infection and symptomatic treatment. The choice of empirical antibiotics was mainly based on characteristics of immune and infection, covering bacteria, fungi and pneumocystis. Intravenous immunoglobulin was often infused. However, most patients (5/7) have died or given up on treatment without transplantation due to severe infection at early stages of the disease. Only P1 received a HLA-matched unrelated HSCT using umbilical cord blood at 29 months. Fludarabine and busulfex were undertaken as the conditioning regimen，cyclosporine A (CsA) as GvHD prophylaxis. Unfortunately, he also died half a month later because of sepsis and respiration-circulation failure without successful engraftment.

Discussion

DNA ligase IV deficiency is a rare primary immunodeficiency that was first reported by Plowman in 1990 [29]. Since then, 54 patients (43 non-Chinese and 11 Chinese) have been already reported in PubMed Medline with a broad spectrum of clinical features [3-26]. Now, we described the clinical and laboratory features of 7 patients with *LIG4* mutations in a Chinese cohort.

In our study, no obvious family history was found, except for P2 and P5. Both mothers had abnormal pregnancies with embryo growth arrest. DNA ligase IV is essential for embryonic viability that knockout mice died at an early embryonic stage [30]. In this cohort, six patients presented with microcephaly, and all seven had growth restriction, which confirms that these two manifestations are the most prominent features of LIG4 syndrome as reported before. Therefore, LIG4 syndrome could be considered when patients present with repeated infection and head circumference more than 3 SD below the population mean. Developmental delay also occurred in two of our patients. In particular, the impairment in expressive language skills was predominant in LIG4 patients [4]. It is necessary to perform early intelligence assessment on patients with microcephaly even if head MRI showed no apparent abnormality.

Even though facial dysmorphism as “bird-like” or “Seckel syndrome-like” is always observed in LIG4 patients, none of our patients were described in the medical history. Other symptoms, including thrombocytopenia, leukopenia, pancytopenia, skin lesions, were all observed in our patients, except for bone abnormalities. The routine blood analysis revealed a profound decrease in thrombocytes in 3 of our patients, and one patient manifested as pancytopenia at the age of 2 years; this patient was suspected of having “myelodysplastic syndromes” (MDS) based on a bone marrow aspiration smear. Most of the patients were at very young age so that thrombocytopenia might be more evident than cytopenia. Pancytopenia can mark progression to bone marrow failure, and MDS have been reported previously [12]. The pathogeny of MDS has not been thoroughly elucidated to date, but reduced telomere length was found in LIG4 syndrome in analogy to other genetically unstable diseases, such as Fanconi anemia and dyskeratosis congenita. We suspected that the accumulation of DSBs in myeloid progenitors led by environmental or infectious triggers and/or autoimmune disorders might contribute to bone marrow failure, as well.

Interestingly, P2 presented with recurrent fever, and colonoscopy indicated extensive intestinal ulceration. To the best of our knowledge, this is the first patient with an IBD-like phenotype. In previously reported cases, only one patient with LIG4 syndrome was documented to present a phenotype mimicking Behcet’s disease (BD) [26] and one female patient who diagnosed with IBD harbored a heterozygous stop-gain (p.R814X) variant in the LIG4 gene [31]. Indeed, IBD-like immunopathology is a common finding in patients with complex defects in T- and B-cell function, such as Wiskott Aldrich syndrome (WAS) and atypical SCID [32] or Omenn syndrome. Though no further information has been found, it seems reasonable to assume LIG4 gene as a candidate gene for IBD [33]. An abnormal immune reaction in LIG4 patients is suspected to be a contributor. Susceptibility to malignancy is common among disorders influencing double-strand DNA break damage repair. Eleven patients have been reported [3, 5-7,9,11,15,18,19, 26], whose median onset age of malignancy is 4 years old. However, no malignancy occurred in our study, possibly because of early death in these patients.

NHEJ is also important for immune development; thus, LIG4 patients can manifest as (severe) combined immunodeficiency. Among the reported cases, about three quarters of patients suffered from recurrent infection with a varying degree of severity. In our case series, recurrent pneumonia and diarrhea are the most common symptoms. Pathogens including *Streptococcus*

pneumoniae, *Haemophilus influenzae*, *CMV*, *Candida albicans*, *Salmonella typhimurium*, and *EBV*, have been frequently detected in LIG4 patients. Exhibiting a slight difference, the *Acinetobacter, Parainfluenza virus* and *Salmonella* infections are also prominent. The immunological detection shows a profound T (especially CD4+ T) and B lymphocytopenia with hypogammaglobulinemia in most of our patients. One patient just showed slightly decreased lymphocytes, and we suspect that there might reflect compensatory mechanisms of proliferation of T cells or chimerism, such as maternal-fetal cellular trafficking. Further studies revealed that the proportion of naive CD4+ and CD8+ T cells was markedly decreased, while memory T cells was increased, which was in accordance with the three siblings reported by Felgentreff et al. [21] The decreased naive T cell may lead to ineffective resistance to primary infection.

Nearly eighty-five percent of reported patients carry compound heterozygous mutations in LIG4 gene, which the most frequent alleles are R814X and K424FS in the non-Chinese cases [3]. The K424FS causes a premature stop codon 20 aa downstream and the protein expression level in skin fibroblasts is notably reduced [8]. Truncating mutation R814X, lying at the XRCC4 binding domain, retained 10%~15% residual double-strand ligation activity [6]. The slight difference, however, is that two mutations R278L and K424FS accounting for the majority of Chinese patients [19, 25]. The R278L mutation resides in the vicinity of the ATP-binding site. We predict that the function mechanism may be similar to the previously reported R278H mutation at the same position. Interaction between the ligase and XRCC4 is not affected, but the enzyme–adenylate complex formation is severely impaired, resulting in the ~10% residual ligase activity [34,35]. In all LIG4-mutated patients, the most prevalent genotypes are p.R814X associated with another truncating mutation, especially p.R814X/p.K424RfsX20; while p.K424RfsX20 associated with mutations near active site (K273) is another frequent genotype [23]. The phenotypes of these two genotypes are also a bit different from each other. Interestingly, p.K424RfsX20/p.R278L is only seen in the Chinese population, and two patients with p.K424RfsX20/p.R278H have been reported before in other countries, highlighting the significance of the genotype p.K424RfsX20/p.R278. Compared with p.R814X/p.K424RfsX20, p.K424RfsX20/p.R278 might lead to milder dysmorphism, but more significant IgA/ IgM deficiency. Hypomorphic mutations in ligase IV, such as R278H, may allow normal development but confer marked radiosensitivity, as reported in previous studies [35]. Decreased IgA/IgM levels might be associated with early clinical digestive symptoms, similar to the disease selective IgA deficiency. Moreover, patients with p.K424RfsX20/p.R278 are at higher risk of severe infections. These findings deepen our understanding of genotype-to-phenotype correlations.

The treatment of LIG4 syndrome mainly includes antibiotic prophylaxis, immunoglobulin substitution, transfusion support and avoiding unnecessary exposure to ionizing radiation [1]. Hematopoietic stem cell transplantation (HSCT) has been performed on ten patients [8, 9,12-14,17,22,23,25], and six cases were successful, while others died because of different complications. Notably, microcephaly or neurodevelopmental delay in these patients cannot be cured by HSCT. In addition, hematopoietic stem cell transplantation that requires cytotoxic agents, especially CsA [36], which could induce DSBs and be harmful for patients with this syndrome; therefore, personalized transplant conditioning should be performed carefully on these patients. In our study, only one patient underwent HSCT and died due to severe infection. Others received supportive treatment. Particularly, P2 was treated with glucocorticoid due to persistent fever and IBD. Immunosuppressant was also used in the reported patient presented with a BD-like phenotype.

Conclusions

In summary, we reported the clinical manifestations and treatment of seven Chinese patients with LIG4 syndrome. In addition, the finding of the IBD-like phenotype might expand the phenotypic spectrum of this disease. R278L and K424FS were two common mutations, and p.K424RfsX20/p.R278L was the only genotype seen in the Chinese population. Genotype-to-phenotype correlations was further understood. Genotype p.K424RfsX20/p.R278 might lead to milder dysmorphism, but more significant IgA/ IgM deficiency, compared with p.R814X/p.K424RfsX20. These results may help to characterize the current status of the disease in China.

Abbreviations

BD: Behçet's disease; DSBs: DNA double strand breaks; GvHD: Graft-versus-host disease; HLA: Human leukocyte antigen; HSCT: Haematopoietic stem cell transplant; IBD: Inflammatory bowel disease; IVIG: Intravenous immune globulin; LIG4: DNA Ligase IV; MDS: Myelodysplastic syndromes; NGS: Next-generation sequencing; NHEJ: Nonhomologous end-joining; UCBSCT: Umbilical cord blood stem cell transplantation.

Declarations

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Availability of data and materials

The datasets used and/or analysed during the current study are all included within the article and available from the corresponding author on reasonable request.

Author contributions

Bijun Sun and Qiuyu Chen drafted the manuscript. Ying Wang and Danru Liu completed the experimental section. Jia Hou, Wenjie Wang, Wenjing Ying, Xiaoying Hui, Qinhua Zhou contributed to study design and data collection. Jinqiao Sun and Xiaochuan Wang gave academic feedback and revised the manuscript. All authors have reviewed the final manuscript and agreed to be accountable for the work.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Children's Hospital of Fudan University. The patients and their parents provided written informed consent for enrollment in this study.

Consent for publication

Not applicable.

Competing interests

All the authors declare that they have no competing interests.

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Tables

Table1. Baseline characteristics of patients with *LIG4* mutations

Patients	P1	P2	P3	P4	P5	P6	P7
Age at presentation	11m	1m	1w	4m	1m	14m	6m
Age of diagnosis	21m	18m	15m	6m	3m	28m	38m
Sex	M	M	F	M	M	F	F
Birth Weight	2050g	2770g	2100g	NA	2700g	2550g	2300g
Family history							
Microcephaly							
Facial dysmorphism							
Developmental retardation							
Growth restriction							
Clinical presentation	Diarrhea, thrombocytopenia, pneumonia, otitis media, thrush, vitiligo	Pneumonia, canker sores, recurrent fever, diarrhea, intestinal ulceration, impaired liver function	Omphalitis, pneumonia, thrush, leukopenia, canker sores, diarrhea, skin and soft tissue infection	Erythroderma, pneumonia, diarrhea, thrombocytopenia	Eczema, lymphadenectasis	Pneumonia, thrush, canker sores, herpes simplex, diarrhea, pancytopenia	Recurrent upper respiratory tract infection, diarrhea, otitis media, pneumonia, thrombocytopenia, leukopenia
Pathogenic microorganism	Sputamentum: *Acinetobacter baumannii, Parainfluenza virus 3, Acinetobacter pittii, Candida albicans, Staphylococcus haemolyticus, Enterobacter cloacae, Flavobacterium menigosepticum*	Sputamentum: *Respiratory syncytial virus*	Blood: *Staphylococcus hominis*; Excrement: *Salmonella typhimurium*	BALF: *Haemophilus influenzae, Pneumocystis, Flavobacterium menigosepticum*; Sputamentum: *Parainfluenza virus 3*	Negative	Excrement: *Salmonella enteritidis*	Sputamentum: *Pseudomonas aeruginosa*
Treatment	UCBSCT	Anti-infection, steroid	Anti-infection	Anti-infection	Anti-infection	Anti-infection	Anti-infection
Follow-up	Died	Died	Lost	Died	Died	Lost	Alive

The primary symptom was listed at first in the table of clinical presention.

M: Male; F:Female; NA: no available; BALF:brocho-alveolar larage fluid; UCBSCT:Umbilical cord blood stem cell transplantation

Table2. Immune index of patients with *LIG4* mutations

Patients	P1	P2	P3	P4	P5	P6	P7
Age (m)	26m	17m	13m	6m	2m	15m	35m
Sex	M	M	F	M	M	F	F
WBC (cells/ul)	3800↓	4000	1700↓	15700	11100	2100↓	1700↓
NEUT (cells/ul)	3360	2870	680↓	14210	3650	490↓	790↓
CD19 (cells/ul)	46.69(6.86%)↓	11.33(9.84%)↓	7.22(2.41%)↓	5.04(0.79%)↓	5.51(0.21%)↓	14.63(0.9%)↓	1.43(0.73%)↓
Naive B (%)	80.0	64.5↓	14.0↓	NA	NA	2.2↓	76.3
Memory B (%)	20.0↑	26.1↑	20.4↑	NA	NA	0.0↓	7.9
Transitional B (%)	0.0↓	10.0	6.4	NA	NA	0.7↓	21.1↑
Plasmablasts (%)	20.0↑	28.7↑	35↑	NA	NA	0.0↓	10.5↑
CD3 (cells/ul)	585.2(86.04%)↓	49.1(42.62%)↓	134.33(44.78%)↓	530.2(82.77%)↓	1934.79(74.5%)↓	999.19(61.53%)↓	146.2(74.45%)↓
CD4 (cells/ul)	138.51(20.37%)↓	32.1(27.87%)↓	41.96(13.99%)↓	344.34(53.75%)↓	782.96(30.15%)↓	113.20(6.97%)↓	58.78(29.93%)↓
CD4 Naive (%)	1.1↓	0.0↓	0.0↓	NA	NA	0.1↓	0.6↓
CD4 CM (%)	62.8↑	56.6↑	59.7↑	NA	NA	53.8↑	83.5↑
CD4 EM (%)	35.2↑	43.1↑	40.3↑	NA	NA	46.0↑	15.9↑
CD8 (cells/ul)	311.25(45.77%)↓	3.78(3.28%)↓	39.69(13.23%)↓	121.34(18.94%)↓	1053.69(40.57%)	676.16(41.64%)	77.42(39.42%)↓
CD8 Naive (%)	0.5↓	4.4↓	6.7↓	NA	NA	0.4↓	1.9↓
CD8 CM (%)	10.4	73.4↑	62.9↑	NA	NA	10.5	88.3↑
CD8 EM (%)	80.0↑	20.2↑	26.4↑	NA	NA	78.3↑	9.6
CD8 TEMRA (%)	9.1	2.0	4.0	NA	NA	10.8	0.3↓
DNT (%)	2.0↑	1.9↑	0.3↓	NA	NA	0.0↓	3.5↑
γδ T (%)	28.8↑	13.4	22.6↑	NA	NA	42.3↑	16.4
CD16CD56 (cells/ul)	35.79(5.26%)↓	52.87(45.90%)↓	150.21(50.07%)↓	99.36(15.51%)↓	631.88(24.33%)	588.36(36.23%)	45.88(23.36%)↓
IgG	12.5*(21d)	8.2*(1d)	7.75	7.6*(5d)	0.83↓	9.68	1.1↓
IgM	2.16	0.35↓	0.11↓	0.28↓	0.03↓	2.57	0.07↓
IgA	0.12	0.54	0.8	0.06↓	0.04↓	0.09	0.1
IgE	23.43	1.56	25.9	2.47	12.5	26	21.16

WBC:White blood count; NEUT:Neutrophil count ; CM: Central memory; EM, Effector memory; TEMRA: Terminal effector memory cytotoxic T cells;

DNT, TCR αβ+ CD4 and CD8 double-negative T cell; NA: Not Available; m: month; d:days; *after intravenous immune globulin (IVIG) therapy

The percentage and numbers of lymphocyte subsets in the peripheral blood reference to the literature [10]

The reference of immunoglobulin

	1-3m	4-6m	12-36m
IgG	2.75-7.50	3.7-8.3	5.52-11.46
IgM	0.05-0.60	0.14-0.5	0.06-0.74
IgA	0.10-0.70	0.33-1.25	0.6-2.12
IgE	<100	<100	<100

Figures

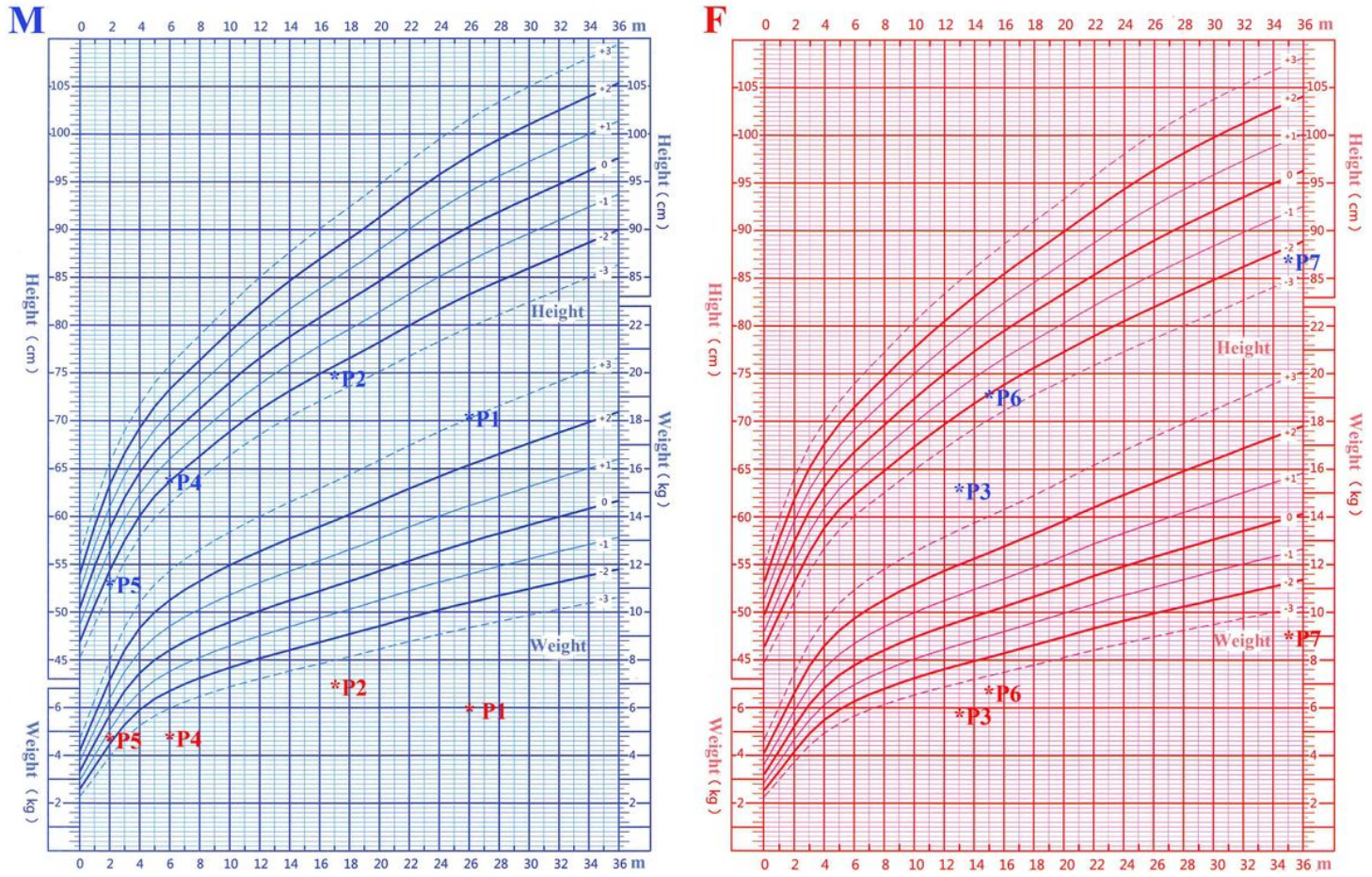

Figure 1

Growth index of patients with LIG4 syndrome compared to healthy children in China. Height is marked with blue, and weight is marked with red. M: male. F: female.

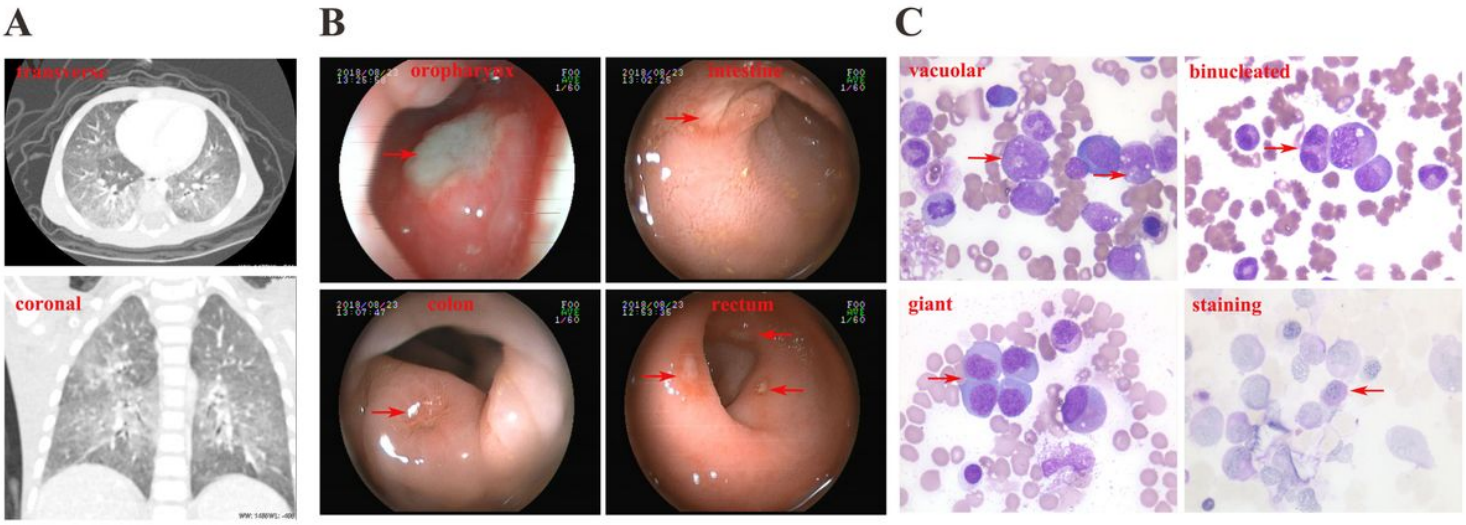

Figure 2

Clinical data of patients with LIG4 syndrome. A: Computed tomography (CT) scan of the chest (P1) showed bilateral lung diffuse lesions with opacification. B: Gastroenterological endoscope examination of P2 demonstrated multiple ulcers of the

oropharynx, small intestine, colon and rectum. C: Morphologic examination of bone marrow (P6) revealed abnormal hematopoiesis in granulocyte and erythroid series to different extents. Vacuolar degeneration, giant or binucleated granulocytes were observed in granulocyte series. Erythroid series were active proliferation, megaloblastic change and occasionally positive PAS staining.