Clinical and genetic analysis of immunodeficiency-related diseases associated with PIK3CD mutations

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Funding source
Special Fund of the Pediatric Medical Coordinated Development Center of Beijing Municipal Administration (No. XTZD20180202); Beijing Municipal Science and Technology Commission (No. Z171100001017050); Scientific Research Common Program of Beijing Municipal Commission of Education (No. KM201710025019); Talent Training Project-Fostering Fund of National Natural Science Foundation of Beijing Children’s Hospital, Capital Medical University (No. GPY201713).

Received: 13 September, 2018; Accepted: 12 December, 2018

INTRODUCTION

The phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit delta gene (PIK3CD), associated with primary immunodeficiency disease (PID), encodes the p110δ catalytic subunit and is predominantly expressed in immune cells such as lymphocytes. The p110δ together with the p85α regulatory subunit form the heterodimeric lipid kinase PI3Kδ, which regulates immune cell metabolism, survival, proliferation, and function through the phosphoinositide 3-kinase (PI3K)-AKT-mammalian target of rapamycin signaling pathway. Several studies have confirmed that a p110δ deletion is strongly associated with the occurrence of many immune diseases, such as inflammatory bowel disease. Moreover, Angulo et al showed that the gain of function (GOF) PIK3CD mutation induced p110δ-activating mutation causing senescent T cells, lymphadenopathy and immunodeficiency (PASLI) disease through defects in the immune function of T and B cells. PASLI is also known as activated PI3K δ syndrome (APDS). Hartman et al reported that the same mutation in PIK3CD could also trigger primary sclerosing cholangitis (PSC).

Currently, fewer than 100 cases with PIK3CD mutations have been reported worldwide, mainly association with PASLI, and the major mutation site is E1021K. In this study, we retrospectively summarized the data of four children from our hospital with PIK3CD mutations, and analyzed their clinical characteristics, genotypes, treatments, and prognoses to improve the knowledge of PIK3CD mutations and related diseases.

CASE REPORT

Mutation analysis

Four patients were enrolled in our study. Three were male and one was female, with a mean age at diagnosis

DOI: 10.1002/ped4.12101

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of 3.98 years (range, 0.6–8.4 years). The four children came from four unrelated Chinese families with no family history of genetic disease. The whole exome sequencing was performed by the second-generation sequencing technology. All children were shown to carry a dominantly inherited \textit{PIK3CD} heterozygous mutation by DNA sequencing analysis. Patients 1, 2, and 3 all carried c.3061G>A (p.E1021K), which is located in the kinase domain of p110δ and is a common pathogenic GOF mutation. These were all spontaneous mutations, and there was no variation in the same sites of their parents. The mutation site in Patient 4 was c.1100C>T (p.S367L), which is found in the C2 domain of the p110δ protein. This mutation was inherited from the patient’s father and is novel. Its pathogenicity is uncertain because the patient’s father is asymptomatic (Figure 1 and Table 1).

Patient 4 also has a recessively inherited heterozygous \textit{RAB27A} mutation (c.11G>T) inherited from his mother. \textit{RAB27A} is linked to PID-associated hemophagocytic lymphohistiocytosis (HLH). However, because the patient’s mother is asymptomatic, the mutation pathogenicity remains indefinite.

Clinical features

Patient 1, a boy aged 8 years and 4 months, presented with persistent lymphadenopathy and splenomegaly accompanied by recurrent fever for more than 3 months. He had been diagnosed with infectious mononucleosis, and was administered ganciclovir and dexamethasone which relieved his symptoms. However, he relapsed many times after stopping the medication and his condition persisted. Our physical and laboratory examination revealed greatly enlarged submaxillary lymph nodes, while hepatosplenomegaly and multiple abnormal lymph nodes in the abdomen and pelvis were detected by ultrasonography (Table 2). The patient had no history of pneumonia. Routine blood testing showed a decreased complete blood cell count. The peripheral blood Epstein Barr virus (EBV) antibody profile indicated a reactivation of EBV infection, and the EBV DNA load in the plasma was increased to \(2.39 \times 10^4\) copies/mL, which was sustained at a high level during the clinical course (Table 3). Bone marrow biopsy excluded the possibility of malignancy. A biopsy of the neck lymph nodes showed the expression of EBV-encoded small RNAs, suggestive of a T cell lymphoproliferative disease. An immunological assay revealed a reversed CD4/CD8 T cell ratio (0.49%), and the absolute number of CD3 CD4+ T cells, CD3 CD8+ T cells, and CD3 CD19+ B cells was remarkably decreased. Additionally, the level of serum immunoglobulin (Ig)M was elevated (2.39 g/L) while IgA was decreased (Table 4).

Patient 2, a 6-month-old baby girl, presented with crying, hepatosplenomegaly, intermittent fever, and developmental retardation for more than 3 months. Our examination revealed a persistent pustule after vaccination on her left arm, and multiple red rashes over her entire body. Lagerhans cell histiocytosis was excluded after skin biopsy. Hepatosplenomegaly and multiple lymphadenopathy in the axilla were detected (Table 2). The patient had a history of repeated pneumonia, and severe pneumonia with respiratory failure was evident on examination. Her white blood cell count, neutrophils, and hemoglobin were greatly decreased (Table 3). Both EBV antibody and EBV DNA levels in the plasma were negative. However, cytomegalovirus (CMV)-IgM and IgG in the peripheral blood were positive, and the CMV
TABLE 2  Main clinical manifestations of four patients with PIK3CD mutations

| Characteristic                              | Patient 1 | Patient 2 | Patient 3 | Patient 4 |
|---------------------------------------------|-----------|-----------|-----------|-----------|
| Age (year)/gender                           | 8.4/Male  | 0.6/Female| 1.7/Male  | 5.1/Male  |
| Recurrent fever                             | Yes       | Yes       | Yes       | Yes       |
| Hepatomegaly (size, under the rib)          | 3 cm      | 4 cm      | 3 cm      | 4 cm      |
| Splenomegaly (size, under the rib)          | 7 cm      | 7.5 cm    | 9 cm      | 2 cm      |
| Lymphadenectasis (site, size)               | submaxill, 3 cm × 4 cm | axilla, 3 cm × 3 cm | neck, 1 cm × 1 cm | - |
| Lymph node biopsy (site, diagnosis)         | Neck      | -         | T lymphoproliferative disease | Neck |
| Recurrent pneumonia                         | No        | Yes       | Yes       | Yes       |
| Respiratory infections                       | No        | Yes       | Yes       | Yes       |
| Anemia                                      | Yes       | Yes       | Yes       | Yes       |
| Rash                                        | No        | Yes       | No        | No        |
| Developmental retardation                   | No        | Yes       | No        | No        |
| Susceptibility to EBV or CMV infection      | Yes       | Yes       | Yes       | Yes       |

EBV, Epstein-Barr virus; CMV, cytomegalovirus.

TABLE 3  Routine blood test and etiology data of four patients with PIK3CD mutations

| Patient | WBC (× 10^9/L) | Hemoglobin (g/L) | Platelet (count/μL) | Neutrophil (count/μL) | CRP (mg/L) | Virus | Plasma virus DNA (copies/mL) | Virus antibody |
|---------|----------------|------------------|--------------------|-----------------------|-----------|-------|-----------------------------|----------------|
|         |                |                  |                    |                       |           |       |                             |                |
| 1       | 2.48           | 97               | 90                 | 1.40                  | 8         | EBV   | 2.39 × 10⁴                  | + + low        |
| 2       | 16.20          | 81               | 261                | 10.10                 | 106       | CMV   | 3.65 × 10⁵                  | - - -        |
| 3       | 0.78           | 63               | 75                 | 0.27                  | 64        | EBV   | 3.89 × 10⁴                  | + + high      |
| 4       | 3.57           | 104              | 30                 | 0.42                  | 31        | EBV   | 1.76 × 10⁵                  | + + high      |

WBC, white blood cell; EBV, Epstein-Barr virus; CMV, cytomegalovirus; CRP, C-reactive protein; CA, capsid antigen; EA, early antigen; NA, nuclear antigen.

TABLE 4  Immunological data of four patients with PIK3CD mutations

| Patient | CD3⁺ count/μL | CD3⁺ CD4⁺ count/μL | CD3⁺ CD8⁺ count/μL | CD3⁺ CD19⁺ count/μL | CD16¹⁶⁺ CD56⁺ count/μL | CD4⁺/CD8⁺ % | CD4⁺/CD19⁺ % | CD16¹⁶⁺/CD56⁺ % | Immunoglobulin (g/L) |
|---------|---------------|--------------------|--------------------|---------------------|------------------------|-------------|-------------|-----------------|---------------------|
|         |               |                    |                    |                     |                        |             |             |                 | IgG                 |
| 1       | 410           | 50.7               | 120                | 14.8                | 240                    | 30.4        | 70          | 8.5             | 17.70               |
| 2       | 3400          | 67.1               | 1980               | 38.9                | 1550                   | 30.4        | 610         | 11.9            | 18.30               |
| 3       | 280           | 76.3               | 190                | 50.3                | 90                     | 24.4        | 6           | 4.6             | 2.49                |
| 4       | 3160          | 99.7               | 57                 | 1.8                 | 3090                   | 97.6        | 6           | 0.2             | 15.60               |

WBC, white blood cell; EBV, Epstein-Barr virus; CMV, cytomegalovirus; CRP, C-reactive protein; CA, capsid antigen; EA, early antigen; NA, nuclear antigen.
DNA concentration in the plasma was increased to 3650 copies/mL, suggestive of an active CMV infection. The subcutaneous tuberculin test was positive (++), and mycobacterium tuberculosis was found in the sputum. An immunological assay showed the absolute number and proportion of each lymphocyte subgroup were normal; however, the levels of both serum IgM (3.69 g/L) and IgG (18.3 g/L) were increased (Table 4).

Patient 3, a boy aged 1 year and 7 months, presented with hepatosplenomegaly and multiple cervical masses for unknown reasons 2 months after birth; a biopsy test at that time suggested inflammatory lesions. His condition persisted for 1 year and 5 months, during which routine blood tests repeatedly showed hypohemoglobin and thrombocytopenia. He had a history of repeated cough and pneumonia, and lung computed tomography (CT) indicated severe pneumonia. His whole blood cell count was greatly decreased (Table 3). Moreover, his bilirubin levels and reticulocytes were clearly increased and the Coombs test was positive. Initially, he was suspected to have autoimmune hemolytic anemia. However, retrospective analysis suggested this might be secondary to EBV infection because both the extractable nuclear antigen antibody and antinuclear antibody tests were negative. The EBV antibody profile of his peripheral blood was indicative of reactivation of EBV infection, and the EBV DNA load in the plasma was $3.89 \times 10^6$ copies/mL, which was sustained at a high level during the clinical course (Table 3). An immunological assay revealed the absolute number of CD3+ CD4+ T cells, CD3+ CD8+ T cells, and CD3+ CD19+ B cells was greatly decreased. However, the level of IgG was greatly elevated in a setting of a low number of B cells. This may be because the patient had received IgG supplementation when the titers were measured (Table 4).

**Diagnosis and treatment**

All four cases presented with repeated fever, hepatosplenomegaly, and blood cell abnormalities as the initial manifestation. Patient 1 showed typical characteristics of chronic active Epstein-Barr virus (CAEBV) infection with no definite reason during his clinical course, so was diagnosed with CAEBV. He received ganciclovir and pegaspargase, liposomal doxorubicin, etoposide, and methylprednisolone (L-DEP) chemotherapy, but had an unfavorable prognosis. Subsequently, he was detected with a pathogenic PIK3CD mutation, and was finally diagnosed with PASLI. Eventually, he underwent haploid allogeneic hematopoietic stem cell transplantation (allo-HSCT) and has remained well 1 year later.

Patient 2 was diagnosed with PASLI and pulmonary tuberculosis. She was treated with ganciclovir, gammaglobulin, and rapamycin for immunodeficiency disease; she was also given rifampicin and isoniazid against tuberculosis infection. During her follow-up, hepatosplenomegaly, lymphadenopathy, and respiratory infections were greatly improved.

Patient 3 had a chronic repeated condition, and was also diagnosed with CAEBV during the early course. For financial reasons, he only received ganciclovir and dexamethasone therapy. This improved his hepatosplenomegaly, lymphadenopathy, and respiratory infections were greatly improved.

Patient 4 was diagnosed with EBV-related T lymphoproliferative disease complicated by HLH. The HLH 2004 chemotherapy regimen was initially applied, but his condition was not well controlled. He then received L-DEP regimen treatment but unfortunately relapsed. Finally, he underwent allo-HSCT and achieved complete remission.

**DISCUSSION**

PI3Kδ, a class 1 PI3K isoform, is a heterodimer comprising a p110α catalytic subunit and a p85 regulatory subunit. PI3Kδ is expressed predominantly in leukocytes and plays an important role in their proliferation, survival, and activation. PIK3CD encodes the catalytic subunit p110δ, a protein of 1044 amino acids with at least five
domains identified by structure and function: an adaptor-binding domain (ABD), a Ras GTPase binding domain (RBD), a C2 domain, a helical domain, and a lipid kinase domain with amino (N) and carboxyl (C) side lobes (Figure 1). Heterozygous GOF variants in PIK3CD can enhance the catalytic activity of p110δ and subsequently increase PI3Kδ basal intracellular signaling in lymphocytes. Overactivation of PI3Kδ and its downstream signaling can lead to a range of B and T cell developmental and functional defects that compromise host defense, leading to clinical manifestations of immunodeficiency.1,6

A total of nine pathogenic GOF variants have been identified in PIK3CD across a range of continents and ethnicities: N333K,6 R405C,7 and C416R8 in the C domain; E81K2 in the ABD domain and G124D9 between two helices in the ABD–RBD linker; E525K and E525A9 in the helical domain; and R929C10 and E1021K11 in the C-terminal lobe of the kinase domain, of which E1021K appears to be a mutation hotspot (Figure 1). These variants cause overactivation of PI3Kδ and the subsequent downstream pathway in two ways depending on the mutation site: they either obstruct binding of the p110δ subunit with the regulatory p85α subunit domain to reduce p85α regulation, such as the E525K and N334K mutation, or they directly enhance recruitment of p110δ to the plasma membrane and increase its catalytic activity, such as the E1021K mutation.6,12,13

PASLI is an autosomal dominant primary immunodeficiency caused by GOF mutations in PIK3CD. It often occurs in young children and is characterized by recurrent sinopulmonary infections with associated lung damage, susceptibility to EBV and cytomegalovirus, hepatosplenomegaly, and lymphoproliferative disease. The main immunologic features are decreased numbers of CD4+ T cells and B cells especially naive CD4+ T cells and naive B cells, increased numbers of transitional B cells, increased IgM levels, and/or decreased IgG. Few cases of PASLI have been reported worldwide and there are no unified clinical diagnosis criteria, so the diagnosis depends mainly on genetic testing.

In this study, we describe three children with the same heterozygous GOF mutation (E1021K) of PIK3CD diagnosed with PASLI. The clinical characteristics of the three children were quite variable despite carrying the same pathogenic mutation, which is similar to the findings of a large patient cohort study report.14 It is worth noting that both Patient 1 and Patient 3 mainly manifested as CAEBV during their clinical course. Indeed, both were diagnosed with CAEBV until the results of genetic testing were obtained, suggesting that CAEBV may be one of the clinical manifestations of PASLI in some cases. Furthermore, the etiology of CAEBV is unknown and this is the first report of PIK3CD mutations in CAEBV, which implies that immune deficiencies caused by PIK3CD mutations are likely to be associated with the pathogenesis of CAEBV. Several cases have been reported in which mutations of STXBP2,15 PRF1,16 JAK317 and GATA218 lead to CAEBV. Similar to PIK3CD, these genes are essential for regulating lymphocyte activation, proliferation, or function, and variants of these genes are closely associated with many primary immunodeficiency diseases. Hartman et al reported that the PIK3CD E1021K mutation could lead to PSC, the common complication of high IgM syndrome, which is also a primary immunodeficiency.5 Taken together, these findings indicated that although most variations of PIK3CD are associated with PASLI, the immune deficiency manifestations caused by PIK3CD mutations may be diverse, and their genetic and immunological mechanisms deserve further investigation.

We also reported a patient (Patient 4) carrying a new heterozygous variant (S367L) of PIK3CD. He had similar clinical manifestations to PASLI, including hematocytopenia, hepatosplenomegaly, EBV virus susceptibility, and lymphoproliferation. However, because this mutation was inherited from his asymptomatic father, it was not sufficient to diagnose PASLI. Compared with the other three children in our study with known pathogenic mutations, the condition of Patient 4 was urgent and progressed rapidly. He also presented with distinct characteristics of T lymphoproliferative disease. Therefore, he was diagnosed with EBV-associated T lymphoproliferative disease combined with HLH. Interestingly, we noticed that the NK cell activity of Patient 4 was reduced in multiple examinations. Moreover, because he was more likely to relapse after chemotherapy, he eventually underwent hematopoietic stem cell transplantation. These findings suggest the possibility of genetic abnormality in this patient. Ultimately, however, only heterozygous mutations of PIK3CD (p.S367L) and RAB27A (p.G4V) were detected, which were inherited from his father and mother, respectively. Although both mutations were predicted to be deleterious by protein structure prediction software, neither of his parents showed similar symptoms to him.

With increasing awareness of HLH, more studies have suggested that it has digenic inheritance. As such, the combination of concurrent partial defects in two or more genes gives rise to a clinical phenotype, whereas a heterozygous state in either of the genes alone results in a less severe phenotype or none at all.19-21 Therefore, in this case, Patient 4 may have carried two hypomorphic genetic defects in PIK3CD and RAB27A, which could have contributed in the development of HLH when he was challenged by viral infection and other stresses. This may also explain the fact that neither parent had relevant clinical manifestations despite carrying one of the same mutations. We hope that this case report will provide clinicians with an insight into possible pathogenic mechanisms of this mutation, although further research is
required to define the functional effects of these genetic alterations.

CONFLICT OF INTEREST

The authors declare no potential conflicts of interest.

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How to cite this article: Zhang Q, Ma H, Ma J, et al. Clinical and genetic analysis of immunodeficiency-related diseases associated with PIK3CD mutations. *Pediatr Invest.* 2018;2:257-262. https://doi.org/10.1002/ped4.12101