Pediatric Rheumatology

CASE REPORT

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Case report: novel *NFKB2* variant associated with pediatric eosinophilic granulomatosis with polyangiitis (EGPA) in the COVID-19 pandemic

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Abstract

Background Childhood-onset eosinophilic granulomatosis with polyangiitis (cEGPA) is a rare type of systemic autoimmune disorder. Variants in the *NFKB2* gene can manifest as common variable immunodeficiency or combined immunodeficiency, often accompanied by autoimmunity and ectodermal dysplasia. Here, we report a case of a Chinese patient who carries *NFKB2* variants that coexist with cEGPA, a novel combination which, to our knowledge, has not been previously published.

Case presentation We reported a 9-year and 10-month-old girl who presented with cough, wheezing, dyspnea, hypereosinophilia, and vasculitis. Notably, she had significant bilateral pulmonary interstitial lesions. We performed metagenomic next-generation sequencing (mNGS), bronchoscopy and immunological analysis. She was considered to have refractory cEGPA after six months of corticosteroid and immunosuppressive treatment. Tapering off corticosteroids posed a challenge, and multiple immunosuppressive agents were ineffective. Our patient suffered from recurrent fever, wheezing, dyspnea and perianal abscess, along with life-threatening infections, including pneumocystis jirovecii pneumonia (PJP) and severe coronavirus disease 2019 (COVID-19) pneumonia during the pandemic. Her cytokines and inflammatory markers showed a profound collapse. She developed significant hypoxemia, which necessitated mechanical ventilation. Primary immunodeficiency gene panel testing revealed a novel de novo variant in *NFKB2* (c.2578 + 2 dup) that was classified as pathogenic. Despite treatment with antibacterial, antiviral, and antifungal agents, biologics, and plasma exchange, she ultimately succumbed to respiratory failure.

Conclusions This case report establishes a novel link between *NFKB2* variants and EGPA, particularly in the context of the COVID-19 pandemic. This study expands the spectrum of *NFKB2* variants and vividly illustrates the complex interrelationships among autoimmunity, infection, and immunodeficiency.

Keywords EGPA, Churg-Strauss syndrome, Childhood-onset eosinophilic granulomatosis with polyangiitis, *NFKB2*, Inborn errors of immunity, COVID-19

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Background

Eosinophilic granulomatosis with polyangiitis (EGPA), also known as Churg-Strauss syndrome, is a rare form of anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) [1]. Childhood-onset EGPA (cEGPA) accounts for less than 2% of pediatric vasculitis cases [2]. Clinical manifestations of EGPA include significant eosinophilia, asthma, and necrotizing vasculitis involve in various organs [3]. Asthma, a cardinal feature of EGPA, can affect 95% to 100% of patients, with the pulmonary region being the most severely affected [4]. The cornerstone therapy for EGPA is corticosteroids. Immunosuppressants such as cyclophosphamide (CYC), azathioprine (AZA), and methotrexate (MTX), plasma exchange, and intravenous immunoglobulins (IVIG) are also used in the conventional treatment of pediatric EGPA [5]. Notably, isolated pediatric cases of EGPA have been successfully treated with emerging targeted therapies, such as dupilumab and mepolizumab [6–8].

NFKB2 is a vital regulator in noncanonical NF-κB pathways, playing key roles in immune processes. Germline *NFKB2* mutations affect the phosphorylation of p100 and the nuclear translocation of p52 [9]. Three types of autosomal-dominant inborn errors of *NFKB2* variants have been identified, $p52^{LOF}$/IκBδ^{LOF}, $p52^{GOF}$/IκBδ^{LOF}, and $p52^{LOF}$/IκBδ^{GOF} [10]. According to previous reports, patients with *NFKB2* ($p52^{LOF}$/IκBδ^{GOF}) have lower memory B cells and are more susceptible to viruses, particularly COVID-19 [11]. Mutations in *NFKB2* were associated with common variable immunodeficiency (CVID) and variable autoimmune features, such as adrenocorticotropic hormone (ACTH) insufficiency, pituitary hormone deficiencies, and alopecia [12, 13]. However, EGPA has not been previously reported in patients with *NFKB2* variants.

Pediatric EGPA cases reported in the literature are currently limited. Here, we describe the challenging diagnosis and treatment course of a girl with refractory cEGPA and an *NFKB2* variant. To our knowledge, this is the first reported case of cEGPA associated with an *NFKB2* variant, especially in the context of COVID-19.

Case presentation

A 9.8-year-old girl was hospitalized at our pediatric tertiary care center with recurrent fever, cough, shortness of breath, and subcutaneous nodules lasting approximately seven months. She had been hospitalized for periorbital cellulitis at the age of 2, as well as chronic gastritis and duodenitis at 9 years and 3 months of age. Additionally, she had a history of allergic rhinitis for seven months and asthma for five years without receiving standard treatment.

Outside hospital course and EGPA diagnosis

Due to persistent wheezing, coughing, and shortness of breath, the patient was referred to a specialized pediatric center at the age of 9 years and 3 months. Laboratory tests revealed elevated C-reactive protein (CRP), an increased erythrocyte sedimentation rate (ESR), and severe eosinophilia (an absolute eosinophil count of 1.9×10^9/L, normal range was 0–0.68×10^9/L, with a relative eosinophil count of 10.6%, normal range was 0.5–9%), and a subcutaneous nodule in her right forearm. A chest computed tomography (CT) scan showed multiple lung nodules. Transbronchial lung biopsy (TBLB) showed scattered eosinophilic infiltration in the interstitial tissue. She was diagnosed with EGPA according to the American College of Rheumatology (ACR) 1990 classification criteria. She underwent pulse therapy with methylprednisolone (MP) and CYC (a total of 152 mg/kg, with a weight of 23 kg), followed by a maintenance dose of prednisone (1 mg/kg/day). Budesonide and montelukast were used for her asthma management. At 9 years and 7 months of age, MTX (10 mg/week) with folic acid supplementation was added to her treatment. However, she continued to experience respiratory symptoms, subcutaneous nodules on her right cheek and bilateral forearms, pain in her left leg, herpes labialis, and high fever while tapering of prednisolone (0.75 mg/kg/day). She was subsequently admitted to our hospital. The clinical course is illustrated in Fig. 1.

Routine assessment at our institution

She underwent multisystem examination, including assessments for pulmonary, cardiac, renal, gastrointestinal, cutaneous, and neurological involvement to differentiate infection from refractory EGPA. Physical examination showed sporadic red maculopapules on the skin, particularly on the right upper limb and both lower limbs. Activity restriction and subcutaneous nodules were observed on the left lower leg. Laboratory investigations showed increased CRP (66.8 mg/L), procalcitonin (PCT, 0.29 ng/mL), and ESR (41 mm/h). The IgA level was reduced (0.08 g/L, with the normal range of 0.41-3.95 g/L). Liver and renal function tests, eosinophil leukocytes (0.3%), serum IgE level, anticardiolipin antibody, autoantibodies, and ANCA serologies were all within normal limits.

Systematic evaluation and Pathogens

Pulmonary CT imaging showed bilateral pulmonary interstitial lesions (Figure 1a). Pulmonary function testing revealed mild restrictive ventilatory impairment. The fractional exhaled nitric oxide level was normal (7 ppb). Serum (1, 3)-β-D-glucan (BDG), parainfluenza virus

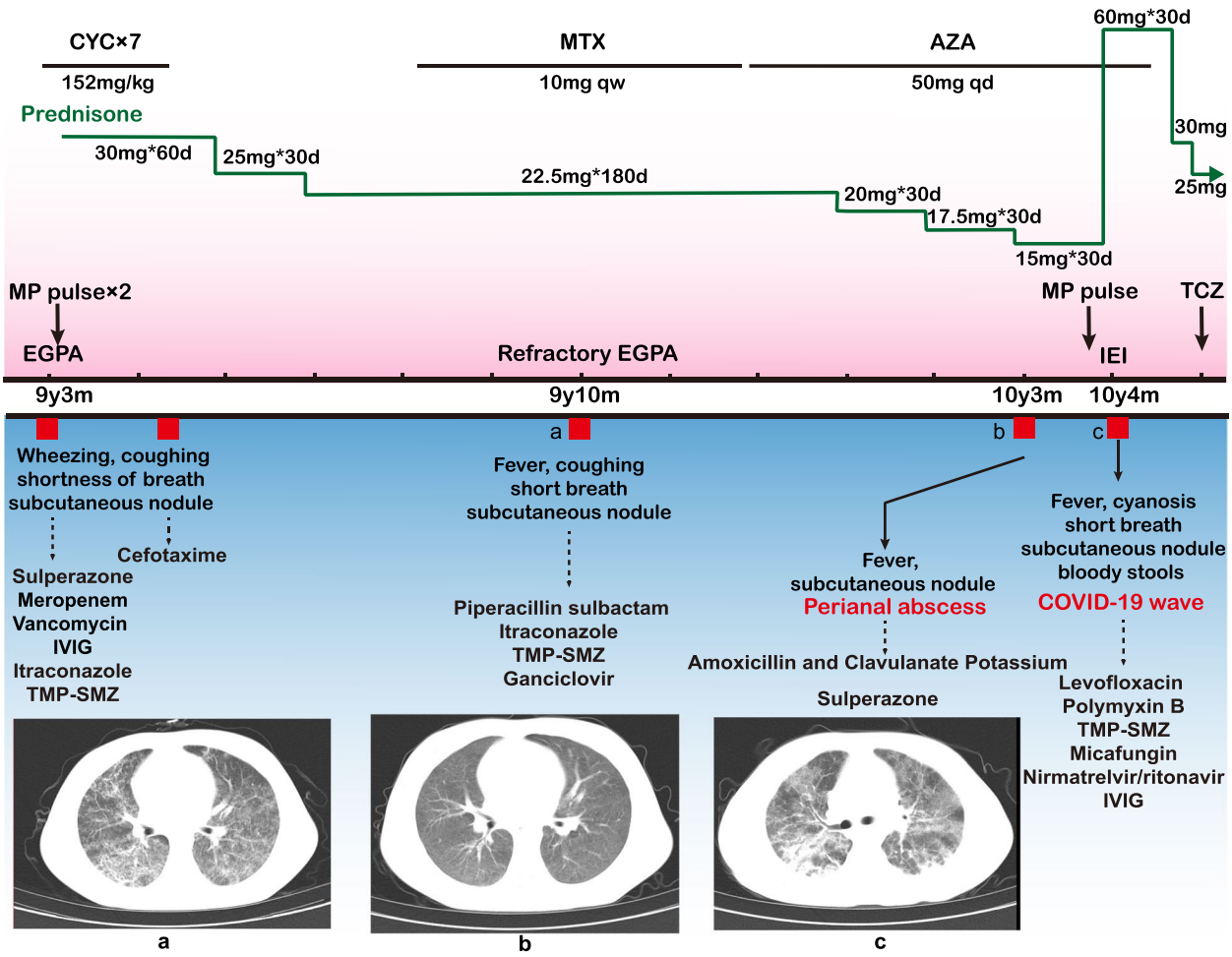

Fig. 1 The clinical course and pulmonary involvement of this patient. **a** Computed tomography (CT) showed bilateral pulmonary interstitial lesions at the age of 9 year and 10 months. **b** Chest CT revealed significant improvement of interstitial lesions in the bilateral lungs. **c** Significant interstitial lung lesions reappear during the COVID-19 pandemic. The upper portion shows the use of glucocorticoids, immunosuppressants, and biologics. The lower part displays the anti-infective situation. The red square indicates the patient requiring hospitalization due to severe infection. AZA: Azathioprine; CYC: Cyclophosphamide; EGPA: Eosinophilic granulomatosis with polyangiitis; IEI: Inborn Error of Immunity; IVIG: Intravenous immunoglobulins; MP: Methylprednisolone; MTX: Methotrexate; TMP-SMZ: Trimethoprim/sulfamethoxazole; TCZ: Tocilizumab

RNA in the throat swab, and cytomegalovirus (CMV) DNA in the blood were all positive. Hemoculture, sputum culture, and fungal culture results were negative. Additionally, the smear, galactomannan (GM) test, Gene Xpert test, Legionella pneumophila DNA, and fungal culture of bronchoalveolar lavage fluid (BALF) were all negative. The patient had high-risk factors for fungal infection, which in combination with pulmonary CT findings and positive BDG, prompted antifungal treatment. She received Piperacillin-Tazobactam (3.3 g, tid, ivgtt), along with itraconazole (0.1 g, qd, po) and trimethoprim/sulfamethoxazole (TMP-SMZ, 720 mg, biw, po) for prophylaxis against fungal infections and pneumocystis jirovecii (P. jirovecii) pneumonia (PJP), respectively.

Then, metagenomic next-generation sequencing (mNGS) of BALF identified Pseudomonas aeruginosa, Acinetobacter baumannii, Streptococcus pneumoniae, Mycoplasma pneumoniae, CMV, human polyomavirus (HPyVs) type 6, and P. jirovecii (Table 1). The patient was diagnosed with PJP. As a result, TMP-SMZ was adjusted to a therapeutic dose (720 mg, q6h, po). She also experienced recurrent herpes labialis. Because CMV was detected in both the BALF and the blood, ganciclovir (160 mg, q12h, ivgtt) was added to her treatment.

Except for the patent foramen ovale (PFO), cardiac ultrasonography and magnetic resonance imaging (MRI) revealed normal findings. A 24-h electrocardiogram showed sinus arrhythmia and premature ventricular

Table 1 The result of metagenomic next-generation sequencing in bronchoalveolar lavage of cEGPA patient

Name	Sequence number	Relative abundance (%)	Coverage (%)
Pseudomonas aeruginosa	79	0.02	0.1
Acinetobacter baumannii	11	0	0.02
Streptococcus pneumoniae	9	0.01	0.03
Mycoplasma pneumoniae	3	0.01	0.03
Cytomegalovirus	930	5.91	20.89
Human polyomavirus type 6	89	27.03	64.49
Pneumocystis jiroveci	225,202	41.19	75.54

contractions (PVC). Troponin I and urinalysis results were normal. She experienced mild abdominal pain below the xiphoid process. A neuroelectrophysiological evaluation of the lower extremities was also normal. Low-molecular-weight heparin (LMWH, 2300 IU/d, ih) was administered to manage small saphenous vein thrombosis in the left calf. She underwent a skin biopsy on her left leg, and histopathological analysis revealed no evidence of vasculitis or eosinophils. Hematological disorders, parasitic infections, pulmonary aspergillosis, hypereosinophilic syndrome (HES), and other rheumatic diseases were ruled out.

Treatment and follow-up

She was managed with prednisone (0.75 mg/kg/d) and MTX. She was identified as having refractory EGPA, as she was unable to achieve remission despite receiving the standard induction regimen for six months. Subsequently, MTX was discontinued, and AZA was started at 50 mg after thiopurine methyltransferase (TPMT) genetic testing. After treatment with AZA and a tapering dose of prednisolone (0.5 mg/kg/day), her rash and subcutaneous nodule improved. We measured the girl's EGPA activity using eosinophil count, CRP and ESR levels (Fig. 2a). At the age of 10 years and 3 months, she rehospitalized due to perianal abscess, accompanied by fever and subcutaneous nodules on her right cheek and bilateral calves (Fig. 2b). Chest CT showed bilateral lung lesions had significantly reduced compared to previous results (Fig. 1b). She received pulses of MP again and the prednisone dose was increased to 2 mg/kg/day.

COVID-19 wave and *NFKB2* variant

The patient was hospitalized in December 2022 due to shortness of breath, cyanosis, and fever, accompanied by bloody stools during the COVID-19 pandemic in China. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) nucleic acids remained persistently positive (Table 2). Along with respiratory distress and failure, this confirmed a diagnosis of COVID-19 pneumonia. Streptococcus pneumoniae, Candida albicans, P. jirovecii and HPyVs type 2 were detected in mNGS of sputum and blood. A chest CT scan showed significant interstitial lung lesions (Fig. 1c). She required high-flow nasal cannula oxygen, but her oxygen saturation level remained critically low at 87%. Consequently, she was admitted to the pediatric intensive care unit (PICU) for mechanical ventilation and inhaled nitric oxide. Immunological analysis showed decreased B cells, IgG and IgA levels (Table 3). CRP and PCT levels were increased (138.8 mg/L and 0.21 ng/ml, respectively). Cytokine analysis showed elevated IL-2R, IL-6, IL-8 and IL-10 (Table 4). In the early stages of the disease, a decrease in in lymphocyte count and various severe infections were observed, likely due to the side effect of immunosuppressive agents. However, as the illness progressed, we considered the possibility of an inborn error of immunity (IEI). Genetic analysis was performed, and the primary immunodeficiency panel identified a de novo variant in intron 22 of the *NFKB2* gene (NM_001077494.3, Chr10:104,161,917–104161918, c.2578+2 dup, hg19) that was classified as pathogenic (PM2_supporting+PVS1+PS2) (Fig. 2c, 2d). This variant has not been reported in ClinVar, PubMed, 1000 Genomes, gnomAD, dbSNP, ClinGen EvRepo or HGMD. We were unable to conduct further immunophenotyping due to her serious condition. In the PICU, she was treated with levofloxacin combined polymyxin B, micafungin and TMP-SMZ, along with nirmatrelvir/ritonavir against SARS-CoV-2, tocilizumab, plasma exchange, IVIG and continuous renal replacement therapy (CRRT). Mepolizumab was not initially administered due to financial and accessibility constraints, and subsequently due to the severity of the disease. Unfortunately, despite these interventions, the patient could not be successfully ventilated and ultimately died.

Discussion and conclusions

We reported a pediatric patient with refractory cEGPA who experienced a life-threatening COVID-19 infection and was ultimately diagnosed with a novel de novo variant in the *NFKB2* gene.

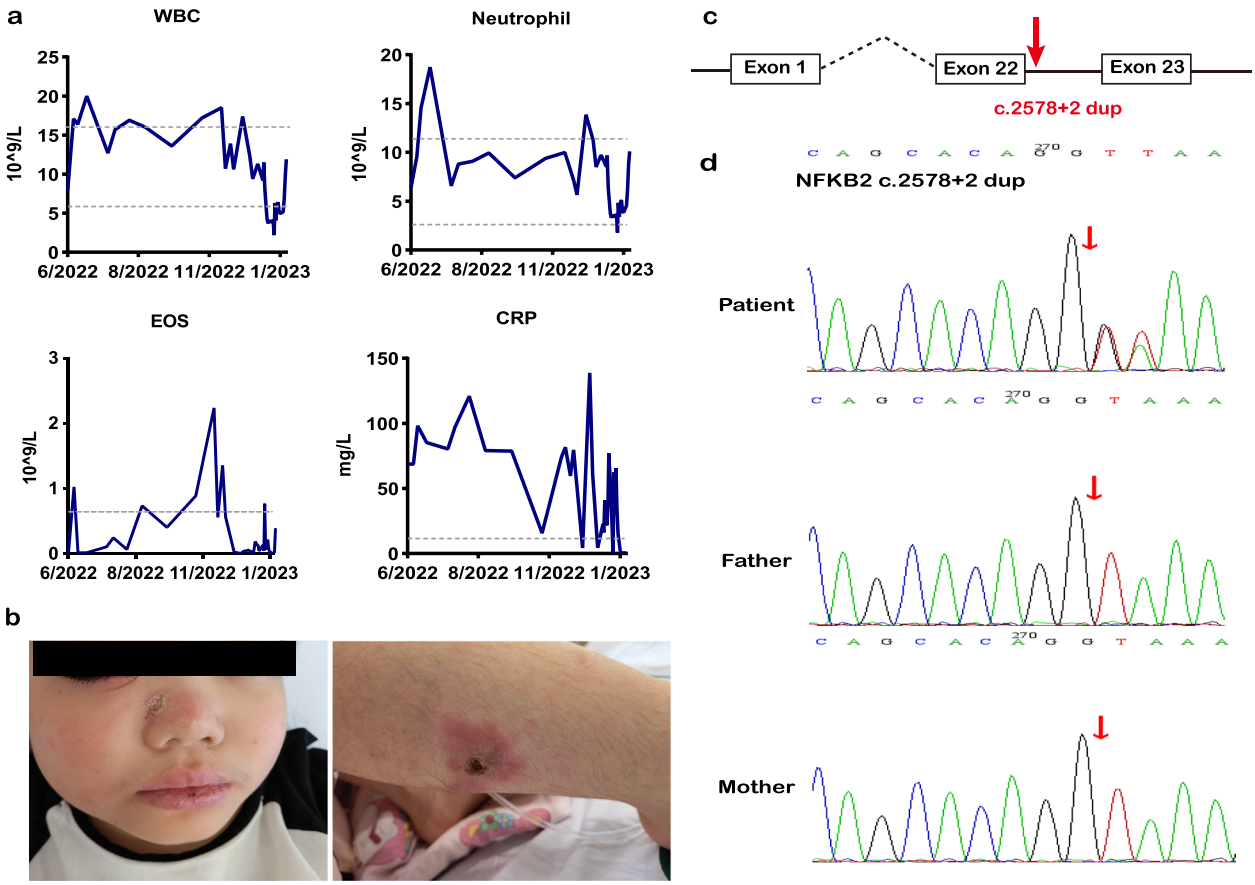

Fig. 2 Hematologic and genetic studies. **a** WBC, CRP, neutrophils and EOS during treatment; **b** Subcutaneous nodules on the patient's right cheek and bilateral lower leg. **c** Illustration of the location of the patient's variant site in the *NFKB2* gene; **d** Sanger sequencing in *NFKB2*. CRP: C-reactive protein; EOS: Eosinophils; WBC: white blood cell

Table 2 SARS-CoV-2 nucleic acid test result of this patient

	Dec 15, 2022	Dec 23, 2022	Jan 2, 2023	Jan 5, 2023	Jan 9, 2023	Jan 12, 2023	Jan 15, 2023	Jan 20, 2023	Normal range
2019-nCOV ORFIa/b(CPA)	(+)	(+)	(+)/30.4	(+)/28.9	(+)/22.5	(+)/30.05	(+)/25.63	(+)/28.43	negative/ ≥ 35
2019-nCOV N Protein(CPA)	(+)	(+)	(+)/29.63	(+)/27.45	(+)/19.7	(+)/27.46	(+)/25.91	(+)/28.9	negative/ ≥ 35

/: CT value of RT-PCR tests

Our patient initially presented with respiratory symptoms, including asthma, recurrent respiratory tract infections, accompanied by elevated eosinophilia, and autoimmune phenomena, such as subcutaneous nodules (a manifestation of vasculitis). EGPA is classified as one of the types of AAV that affect small to medium-sized blood vessels [3]. Currently, no universally accepted diagnostic criteria for EGPA, and the classification criteria are commonly used in practice. The ACR 1990 classification criteria and Lanham criteria are frequently referenced in the literature [1, 14]. The patient exhibited: 1) a history of asthma, 2) elevated peripheral eosinophilia (eosinophilia > 10% or > 1.5 × 10^9/L), 3) pulmonary infiltrates, and 4) cutaneous and venous vasculitis. In this case, scattered eosinophils were present in the lung biopsy, while none were detected in the skin biopsy. The following reasons were considered: 1) due to the influence of specimen collection, no obvious lesion location was sampled, leading to potential false-negative results; 2) eosinophils are highly sensitive to corticosteroids, and the long-term use of steroids at the time of the skin biopsy may have contributed to their absence. Additionally, this patient meets

Table 3 Lymphocyte and immunoglobulin of this patient during the COVID-19 pandemic

	Dec 6, 2022	Dec 21, 2022	Jan 8, 2023	Normal range
CD3%	89.60	91.50	91.00	52 ~ 78
CD3 (×10^9/L)	1.31	1.00	0.23	0.7 ~ 4.2
CD4%	43.30	38.80	28.40	25 ~ 48
CD4 (×10^9/L)	0.63	0.42	0.07	0.3 ~ 2
CD8%	43.70	51.20	61.20	19 ~ 34
CD8 (×10^9/L)	0.64	0.56	0.15	0.3 ~ 1.8
CD4/CD8	1.00 ↓	0.80 ↓	0.50 ↓	1.5 ~ 2
CD19%	6.90 ↓	6.90 ↓	6.50 ↓	10 ~ 31
CD19 (×10^9/L)	0.10	0.08 ↓	0.02 ↓	0.2 ~ 1.6
CD16/56%	2.70	0.90 ↓	1.90 ↓	4 ~ 26
CD16/56 (×10^9/L)	0.04	0.01	< 0.01	0.09 ~ 0.9
IgG(g/L)	—	3.30 ↓	—	5.29 ~ 21.9
IgA(g/L)	—	0.07 ↓	—	0.41 ~ 3.95
IgM(g/L)	—	0.68	—	0.48 ~ 2.26

—: Not detected

↓: Indicates a drop in values

Table 4 Cytokines of this patient

	Jan 6, 2023	Jan 10, 2023	Jan 13, 2023	Jan 17, 2023	Normal range
IL1β	< 5	< 5	< 5	< 5	< 5 pg/ml
IL2R	5041.0	4243.0	2314.0	1473.0	223-710U/ml
IL6	58.1	15.9	82.7	67.6	< 5.9 pg/ml
IL10	154.0	56.2	25.9	24.3	< 9.1 pg/ml
TNFα	19.9	16.4	11.1	7.7	< 8.1 pg/ml
IL8	146.0	308.0	100.0	76.7	< 62 pg/ml

the 2022 ACR/EULAR EGPA classification criteria, achieving a score of + 8 points (+ 3 for obstructive airway disease, + 5 for blood eosinophil count > 1 × 10^9/L) [15]. Differentiating HES from EGPA can be difficult due to their overlapping features of vasculitis and hypereosinophilia. Elevated CRP levels have been reported as a reliable diagnostic biomarker to distinguish HES from ANCA-negative EGPA with asthma, as higher CRP levels are more indicative of EGPA [16]. Our patient consistently presented with elevated CRP, supporting the diagnosis of EGPA. Data on the prevalence of pediatric cEGPA are limited. To date, there have been sporadic case reports and only four large cohort studies reporting on 33, 9, 13, and 14 patients, respectively [2, 17–19]. We reviewed the clinical features and treatment measures of the four cohorts, as shown in Fig. 3. Pediatric cases of EGPA exhibited more severe cardiopulmonary disease, gastrointestinal tract involvement and mortality, but had lower rates of musculoskeletal, renal and peripheral nerve manifestations compared to adults [17, 18]. Consistent with these findings, our patient exhibited involvement of the ear, nose and throat (ENT), pulmonary system, gastrointestinal tract, skin, and potential cardiac involvement, without peripheral nerve or renal disease, but lacked biopsy-confirmed findings in skin. It has been demonstrated that interstitial lung disease (ILD) in AAV is associated with poor outcomes, particularly in patients with microscopic polyangiitis (MPA) [20]. Diffuse alveolar hemorrhage (DAH) is a common and life-threatening manifestation of pulmonary involvement in MPA while the frequency of DAH is rare (4%) and presents with moderate severity in EGPA [4, 21].

Subsequently, a standard therapeutic regimen was initiated, involving glucocorticoids and immunosuppressants. However, during steroids tapering, she experienced recurrent respiratory distress, accompanied by fever and perianal abscess. Following interventions with IVIG and aggressive pulse steroid therapy, her symptoms were improved. Nevertheless, steroid dose reduction remained challenging. Interestingly, prolonged use of steroids and immunosuppressants may develop severe lymphopenia and increase risk of infection [22, 23]. However, recent

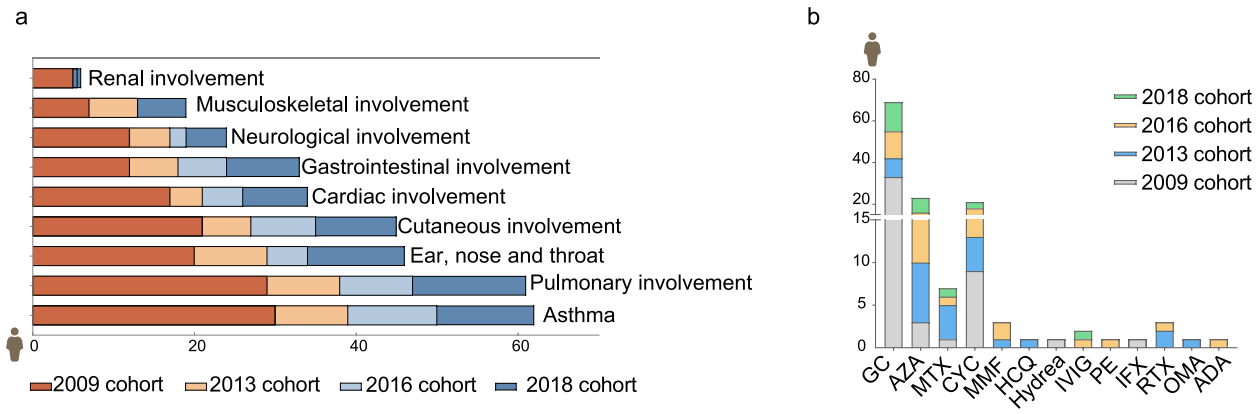

Fig. 3 The summary of four cEGPA cohort. **a** The clinical features of reported cohort. **b** The management strategies of cEGPA patients in 2009, 2013,2016 and 2018 cohort. GC: Glucocorticoids; MMF: Mycophenolate mofetil; HCQ: Hydroxychloroquine; Hydrea: Hydroxyurea; PE: Plasma exchange; IFX: Infliximab; RTX: Rituximab; OMA: Omalizumab; ADA: Adalimumab

studies have demonstrated that in patients diagnosed with both rheumatic diseases and COVID-19, DMARDs or NSAIDs do not elevate the probability of hospitalization [24]. Supporting this finding, Khalil et al. reported that the severity and mortality rates of COVID-19 were not significantly different in patients with systemic autoimmune diseases receiving immunomodulatory therapy compared to the general population [25]. In this case, the patient exhibited more severe respiratory symptoms, interstitial lung disease, and perianal abscess than patients with autoimmune diseases who were also undergoing steroid and immunosuppressive therapy during the COVID-19 pandemic in China. Therefore, we propose that her condition may not be explained by EGPA alone, and IEI should be considered as a diagnosis, despite the absence of a family history of IEI. Ultimately, genetic sequencing validated our hypothesis, indicating the importance of gene sequencing technology in refractory autoimmune diseases. Our patient meets the following criteria for IEI diagnosis: 1) Clinical manifestations: she is susceptible to a wide range of bacterial, viral, and fungal infections, including severe infections such as perianal abscesses, as well as opportunistic infections like PJP. Autoimmune phenomena are also present, such as subcutaneous nodules and rashes. 2) Laboratory tests: both humoral and cellular immunity were reduced. 3) Treatment: The standard immunosuppressants not yield effective results. 4) Genetic testing: gene panel and Sanger sequencing revealed *NFKB2* variants but lacks further pathogenic functional testing. 5) Excluding secondary immunodeficiency induced by immunosuppressants. In summary, the patient is eligible for a diagnosis of IEI coexisting with EGPA.

The patient carries a novel pathogenic variant that was in intron22 of the *NFKB2* gene, c.2578 + 2dup. The variant affects the C-terminal domain of the protein. This alteration may disrupt the phosphorylation sites essential for the degradation of p100 into the mature transcription factor subunit p52. Notably, most reported variants are situated near the critical phosphorylation sites S866 and S870 [26–28].

The non-canonical NF-κB pathway plays a key role in the generation of plasma cells, class switching, and the development of memory B cells. Patients with mutations in *NFKB2* showed the immunological phenotype characterized by reduced class-switched memory B cells, impaired B cells differentiation and hypogammaglobulinemia [29, 30]. Previous reports have also shown impaired T cell proliferation and reduced NK cell cytotoxic activity, which contribute to increased susceptibility to viral infections [31–35]. Consistent with previous findings, serum levels of IgA and IgG in the patient were low, and both B lymphocyte and NK cell counts were reduced. However, we were unable to measure further lymphocyte subpopulations or conduct functional assays because the patient's death from respiratory failure. Mutations in *NFKB2* can lead to CVID, which is often associated with autoimmune manifestations, such as vasculitis. Although a review of *NFKB2* mutations did not find any cases of vasculitis [30], Mac et al. described a female patient with vasculitis-like lesions associated with an *NFKB2* mutation [12]. Dysregulation of the NF-κB signaling pathway can result in excessive production of pro-inflammatory cytokines, including TNF-α and IL-6, which play a key role in the pathogenesis of vasculitis and were markedly elevated in our patient. This suggests that the *NFKB2* mutation may have led to dysregulate NF-κB signaling activation, triggering a cytokine cascade and vasculitis. There is no direct evidence linking *NFKB2* to IL-5. However, activated Th2 cells release eosinophil

chemoattractants like IL-5, which drive the development and maturation of eosinophil, B cell differentiation, and immunoglobulin production. Increased Th17 and decreased Treg cells have been observed in patients with EGPA. *NFKB2* mutations impact the non-canonical NF-κB signaling pathway, affecting inflammatory regulation and B cell growth. This suggests a potential indirect synergistic interaction between *NFKB2* and IL-5, which warrants further research.

The absence of B cells and immunoglobulins increases susceptibility to infections, and an immunocompromised state may heighten the risk of sustained severe SARS-CoV-2 infection [36]. Recent studies indicate that patients with *NFKB2* mutations are vulnerable to viral infections, such as Epstein-Barr Virus (EBV), CMV, herpes virus [37], and particularly SARS-CoV-2 [11]. Moreover, patients with *NFKB2*($p52^{LOF}$/$IκBδ^{GOF}$) variants produce autoantibodies that neutralize type I interferons increasing susceptibility to lethal COVID-19 pneumonia [10, 38]. Our patient showed significant characteristics of viral susceptibility, including herpes labialis, CMV infection, and life-threatening COVID-19 pneumonia. A reported case demonstrated severe SARS-CoV-2 infection associated with *NFKB2* loss-of-function pathogenic variants, consistent with our patient's experience of requiring mechanical ventilation and biologics [39]. However, the outcomes differed between the two patients, possibly due to the absence of opportunistic infections such as PJP in the previously reported case. P. jirovecii and CMV exacerbated the infection during the critical phase of the illness. Additionally, our patient did not receive COVID-19 convalescent plasma (CP), which may be a crucial factor influencing prognosis.

Notably, patients with *NFKB2* variants in previously reported cohorts are predisposed to adrenocortical insufficiency, autoimmune disorders, and ectodermal dysplasia. ACTH deficiency, ectodermal dysplasia, and common variable immunodeficiency associated with *NFKB2* mutations are classified as deficient anterior pituitary with variable immune deficiency (DAVID) syndrome [40]. Therefore, screening for anterior and posterior pituitary deficiencies should be conducted in patients carrying *NFKB2* variants [12]. In this case, this patient exhibits autoimmune susceptibility, such as subcutaneous nodules and asthma, but lacks ectodermal dysplasia and other symptoms such as alopecia, trachyonychia, and hypohidrosis. However, pituitary MRI, and assessments of ACTH and cortisol level were not performed for this patient. Consequently, the presence of ACTH deficiency, growth hormone deficiency, or thyroid-stimulating hormone deficiency remains undetermined.

Differentiating autoimmunity from infection poses a significant challenge for pediatricians, particularly in patients with prolonged use of immunosuppressants [41]. Differentiating EGPA against COVID-19 in the pandemic context presents a diagnostic challenge, due to the similarities in respiratory distress and interstitial pneumonia. But the report showed no poor outcomes in EGPA patients who had COVID-19 [42]. At present, no standardized definitions exist for remission, relapse, or refractory disease specifically for pediatric EGPA. Therefore, we apply the criteria for adults: remission of EGPA is defined as the absence of clinical signs or symptoms of active disease with or without immunosuppressive therapy. The maximal daily dose of prednisone is 5 mg. Relapse of EGPA is defined as the recurrence of clinical signs or symptoms of active disease after a period of remission. Refractory EGPA is defined as unchanged or increased signs, symptoms or other features after a period of standard induction therapy. Active disease is defined as new, persistent, or worsening clinical signs and/or symptoms associated with EGPA, independent of prior organ damage [43, 44]. The patient did not achieve remission throughout the course of the disease. Moreover, the patient displayed no signs of infection (fever, coughing, or shortness of breath) during outpatient follow-up, and no new infectious pathogens were identified. However, her disease remained active, and the glucocorticoid dose was unable to be reduced to the minimum. As a result, her condition was classified as active, refractory EGPA rather than infection-driven disease activity. Consequently, understanding the molecular and genetic mechanisms of IEI is crucial for disease screening and therapeutic strategies. Furthermore, increased vigilance is required for opportunistic infections in the context of IEI. In this case, we utilized multiple diagnostic methods to detect infection, including metagenomic sequencing of blood and BALF. mNGS is a widely applied high-throughput approach for pathogen detection, with the advantages of rapid, convenient and highly sensitive detection [45]. The detection of pediatric pneumonia was enhanced by BALF mNGS compared to conventional microbiological tests (CMTs) [46]. Our BALF mNGS analysis identified a total of seven pathogens, with P. jiroveci showing the highest sequence number, relative abundance and coverage, followed by HPyVs type 6 and CMV. Her clinical symptoms indicate that P. jiroveci and CMV are clinically significant. To the best of our knowledge, this is the first report of severe P. jiroveci infection in cEGPA. As is well known, infections are a frequent complication following immunosuppressive therapy, yet severe infections in EGPA are not common [17]. Despite routine prophylaxis with TMP-SMZ after initiating immunosuppression, she developed severe PJP. Nonetheless, it is essential to exclude background noise from the large number of pathogens detected by mNGS.

This study has certain limitations. The diagnosis of EGPA primarily relies on classification criteria, and no universally accepted diagnostic criteria currently exist for EGPA. Furthermore, due to limitations in specimens' availability and laboratory conditions, further pathogenic functional testing of the *NFKB2* variant was not conducted in this case.

In summary, given the rarity of pediatric EGPA in clinical practice, it is imperative to promptly initiate comprehensive genetic sequencing and immune function assessments in a timely manner for patients who exhibit refractory treatment outcomes. This includes challenges in reducing corticosteroid doses or the development of severe infections, such as perianal abscesses. Additionally, employing multiple methods to search for evidence of infection is essential. Further accumulation of cases is required to facilitate additional research.

We identified a novel pathogenic splicing mutation in the *NFKB2* gene. This is the first report of cEGPA in a patient with an *NFKB2* variant. Our findings may broaden the clinical phenotype of *NFKB2* and heighten understanding of cEGPA heterogeneity. In this case, we learned that refractory cEGPA should be closely monitored for possible IEI, as life-threatening COVID-19 pneumonia was the primary cause of death in the patient with the *NFKB2* variant.

Abbreviations

BDG	(1, 3)-**B**-D-Glucan
ACTH	Adrenocorticotropic Hormone
ACR	American College of Rheumatology
AAVs	Anca-Associated Vasculitis
ANCA	Anti-Neutrophil Cytoplasmic Antibody
AZA	Azathioprine
BALF	Bronchoalveolar Lavage Fluid
cEGPA	Childhood-Onset Eosinophilic Granulomatosis with Polyangiitis
CVID	Common Variable Immunodeficiency
CT	Computed Tomography
CRRT	Continuous Renal Replacement Therapy
CP	Convalescent Plasma
CMTs	Conventional Microbiological Tests
COVID-19	Coronavirus Disease 2019
CRP	C-Reactive Protein
CYC	Cyclophosphamide
CMV	Cytomegalovirus
DAVID	Deficient Anterior Pituitary with Variable Immune Deficiency
DAH	Diffuse Alveolar Hemorrhage
ENT	Ear, Nose and Throat
EGPA	Eosinophilic Granulomatosis with Polyangiitis
EBV	Epstein-Barr Virus
ESR	Erythrocyte Sedimentation Rate
GM	Galactomannan
HES	Hypereosinophilic syndrome
HPyVs	Human Polyomavirus
IEI	Inborn Error of Immunity
ILD	Interstitial Lung Disease
IVIG	Intravenous Immunoglobulins
LMWH	Low-Molecular-Weight Heparin
MRI	Magnetic Resonance Imaging
mNGS	Metagenomic Next-Generation Sequencing
MTX	Methotrexate
MP	Methylprednisolone
MPA	Microscopic Polyangiitis
PFO	Patent Foramen Ovale
PICU	Pediatric Intensive Care Unit
P. jirovecii	Pneumocystis Jirovecii
PJP	Pneumocystis Jirovecii Pneumonia
PVC	Premature Ventricular Contractions
PCT	Procalcitonin
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
TPMT	Thiopurine Methyltransferase
TBLB	Transbronchial Lung Biopsy
TMP-SMZ	Trimethoprim/Sulfamethoxazole

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Authors' contributions
All authors contributed to the study's conception and design. Data collection and analysis was performed by Li Lin and Xin Peng. The first draft of the manuscript was written by Li Lin. Visualization of manuscript was implemented by Li Lin. Supervision and review was performed by Lina Chen, Liqun Dong and Lin Zhong. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability
The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The study conformed with the Declaration of Helsinki's requirements and was given permission by the Institutional Review Board/Ethics Committee related to West China Second University Hospital, Sichuan University (2020111). Informed consent was obtained from the guardians of the patient.

Consent for publication
The family has consented to the submission of the case report to the journal.

Competing interests
The authors declare no competing interests.

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References

1. Emmi G, Bettiol A, Gelain E, Bajema IM, Berti A, Burns S, et al. Evidence-Based Guideline for the diagnosis and management of eosinophilic granulomatosis with polyangiitis. Nat Rev Rheumatol. 2023;19(6):378–93.
2. Gendelman S, Zeft A, Spalding SJ. Childhood-onset Eosinophilic Granulomatosis with Polyangiitis (formerly Churg-Strauss Syndrome): a contemporary single-center cohort. J Rheumatol. 2013;40(6):929–35.
3. White J, Dubey S. Eosinophilic granulomatosis with polyangiitis: A review. Autoimmun Rev. 2023;22(1): 103219.
4. Nguyen Y, Guillevin L. Eosinophilic Granulomatosis with Polyangiitis (Churg–Strauss). Semin Respir Crit Care Med. 2018;39(04):471–81.
5. Raffray L, Guillevin L. Treatment of eosinophilic granulomatosis with polyangiitis: a review. Drugs. 2018;78(8):809–21.

6. Ozola L, Aleksejeva E, Stoldere D, Dāvidsone Z, Šantere R, Grantiņa I, et al. Mepolizumab: Therapeutic strategy for a pediatric patient with eosinophilic granulomatosis with polyangiitis. Pediatr Pulmonol. 2023;58(3):973–9.
7. Galant-Swafford J, Geng B, Leibel S, Akuthota P, Tucker S, Cernelc-Kohan M, et al. Two pediatric cases of ANCA-negative eosinophilic granulomatosis with polyangiitis successfully treated with dupilumab. J Allergy Clin Immunol Pract. 2020;8(10):3643-3646.e1.
8. Joseph MX, Jenkins LE, Wechsler ME, Davis LS. Mepolizumab Treatment of Pediatric Eosinophilic Granulomatosis With Polyangiitis. JAMA Dermatol. 2018;154(4):486.
9. Chen K, Coonrod EM, Kumánovics A, Franks ZF, Durtschi JD, Margraf RL, et al. Germline mutations in NFKB2 implicate the noncanonical NF-κB pathway in the pathogenesis of common variable immunodeficiency. Am J Hum Genet. 2013;93(5):812–24.
10. Le Voyer T, Parent AV, Liu X, Cederholm A, Gervais A, Rosain J, et al. Autoantibodies against type I IFNs in humans with alternative NF-κB pathway deficiency. Nature. 2023;623(7988):803–13.
11. Fathi N, Nirouei M, SalimianRizi Z, Fekrvand S, Abolhassani H, Salami F, et al. Clinical, Immunological, and Genetic Features in Patients with NFKB1 and NFKB2 Mutations: a Systematic Review. J Clin Immunol. 2024;44(7):160.
12. Mac TT, Castinetti F, Bar C, Julia S, Pasquet M, Romanet P, et al. Deficient anterior pituitary with common variable immune deficiency (DAVID syndrome): a new case and literature reports. J Neuroendocrinol. 2023;35(6):e13287.
13. Wirasinha RC, Davies AR, Srivastava M, Sheridan JM, Sng XYX, Delmonte OM, et al. Nfkb2 variants reveal a p100-degradation threshold that defines autoimmune susceptibility. J Exp Med. 2021;218(2):e20200476.
14. Jariwala M, Laxer RM. Childhood GPA, EGPA, and MPA. Clin Immunol. 2020;211:108325.
15. Grayson PC, Ponte C, Suppiah R, Robson JC, Craven A, Judge A, et al. 2022 American College of Rheumatology/European Alliance of Associations for Rheumatology Classification Criteria for Eosinophilic Granulomatosis with Polyangiitis. Ann Rheum Dis. 2022;81(3):309–14.
16. Leurs A, Chenivesse C, Lopez B, Gibier JB, Clément G, Groh M, et al. C-Reactive protein as a diagnostic tool in differential diagnosis of hypereosinophilic syndrome and antineutrophil cytoplasmic antibody-negative eosinophilic granulomatosis with polyangiitis. J Allergy Clin Immunol Pract. 2019;7(4):1347-1351.e3.
17. Fina A, Dubus JC, Tran A, Derelle J, Reix P, Fayon M, et al. Eosinophilic granulomatosis with polyangiitis in children: Data from the French RespiRare® cohort. Pediatr Pulmonol. 2018;53(12):1640–50.
18. Zwerina J, Eger G, Englbrecht M, Manger B, Schett G. Churg-strauss syndrome in childhood: a systematic literature review and clinical comparison with adult patients. Semin Arthritis Rheum. 2009;39(2):108–15.
19. Eleftheriou D, Gale H, Pilkington C, Fenton M, Sebire NJ, Brogan PA. Eosinophilic granulomatosis with polyangiitis in childhood: retrospective experience from a tertiary referral centre in the UK. Rheumatol Oxf Engl. 2016;55(7):1263–72.
20. Kadura S, Raghu G. Antineutrophil cytoplasmic antibody-associated interstitial lung disease: a review. Eur Respir Rev. 2021;30(162):210123.
21. Kambhatla S, Vipparthy S, Manadan AM. Rheumatic diseases associated with alveolar hemorrhage: analysis of the national inpatient sample. Clin Rheumatol. 2023;42(4):1177–83.
22. Da G, J Z, S M, Mm O, As B, K G, et al. Immunosuppression Exposure and Risk of Infection-Related Acute Care Events in Patients With Glomerular Disease: An Observational Cohort Study. Kidney Med. 2022;4(11). Available from: https://pubmed.ncbi.nlm.nih.gov/36339665/. Cited 2024 Oct 16
23. Glück T, Kiefmann B, Grohmann M, Falk W, Straub RH, Schölmerich J. Immune status and risk for infection in patients receiving chronic immunosuppressive therapy. J Rheumatol. 2005;32(8):1473–80.
24. Gianfrancesco M, Hyrich KL, Al-Adely S, Carmona L, Danila MI, Gossec L, et al. Characteristics associated with hospitalisation for COVID-19 in people with rheumatic disease: data from the COVID-19 Global Rheumatology Alliance physician-reported registry. Ann Rheum Dis. 2020;79(7):859–66.
25. Ansarin K, Taghizadieh A, Safiri S, MalekMahdavi A, Ranjbar S, Teymouri S, et al. COVID-19 outcomes in patients with systemic autoimmune diseases treated with immunomodulatory drugs. Ann Rheum Dis. 2022;81(10):e190.
26. Xiao B, Fang D, Liu H, Wang L, Qiu W. A novel NFKB2 mutation in a Chinese patient with DAVID syndrome. Gene. 2019;1(687):319–22.
27. Sundaram K, Ferro M, Hayman G, Ibrahim MAA. Novel NFKB2 Pathogenic Variants in Two Unrelated Patients with Common Variable Immunodeficiency. J Clin Immunol. 2023;43(6):1159–64.
28. Kuehn HS, Bernasconi A, Niemela JE, Almejun MB, Gallego WAF, Goel S, et al. A Nonsense N -Terminus NFKB2 Mutation Leading to Haploinsufficiency in a Patient with a Predominantly Antibody Deficiency. J Clin Immunol. 2020;40(8):1093–101.
29. Lougaris V, Moratto D, Baronio M, Lorenzini T, Rossi S, Gazzurelli L, et al. Early B cell developmental impairment with progressive B cell deficiency in NFKB2 mutated CVID disease without autoimmunity. Clin Immunol Orlando Fla. 2019;205:153–5.
30. Klemann C, Camacho-Ordonez N, Yang L, Eskandarian Z, Rojas-Restrepo JL, Frede N, et al. Clinical and Immunological Phenotype of Patients With Primary Immunodeficiency Due to Damaging Mutations in NFKB2. Front Immunol. 2019;10:297.
31. Slade CA, McLean C, Scerri T, Giang TB, Megaloudis S, Strathmore A, et al. Fatal Enteroviral Encephalitis in a Patient with Common Variable Immunodeficiency Harbouring a Novel Mutation in NFKB2. J Clin Immunol. 2019;39(3):324–35.
32. Shi C, Wang F, Tong A, Zhang XQ, Song HM, Liu ZY, et al. NFKB2 mutation in common variable immunodeficiency and isolated adrenocorticotropic hormone deficiency: A case report and review of literature. Medicine (Baltimore). 2016;95(40): e5081.
33. Montin D, Licciardi F, Giorgio E, Ciolfi A, Pizzi S, Mussa A, et al. Functional evaluation of natural killer cell cytotoxic activity in NFKB2-mutated patients. Immunol Lett. 2018;194:40–3.
34. Bienias M, Gabrielyan A, Geberzahn L, Rösen-Wolff A, Huebner A, Jacobsen EM, et al. More severe than CVID: Combined immunodeficiency due to a novel NFKB2 mutation. Pediatr Allergy Immunol Off Publ Eur Soc Pediatr Allergy Immunol. 2021;32(4):793–7.
35. Kuehn HS, Niemela JE, Sreedhara K, Stoddard JL, Grossman J, Wysocki CA, et al. Novel nonsense gain-of-function NFKB2 mutations associated with a combined immunodeficiency phenotype. Blood. 2017;130(13):1553–64.
36. Choi B, Choudhary MC, Regan J, Sparks JA, Padera RF, Qiu X, et al. Persistence and Evolution of SARS-CoV-2 in an Immunocompromised Host. N Engl J Med. 2020;NEJMc2031364.
37. Parsons K, Cipriano SD, Rosen LB, Browne SK, Walter JE, Stone BL, et al. Severe Facial Herpes Vegetans and Viremia in NFKB2-Deficient Common Variable Immunodeficiency. Front Pediatr. 2019;7:61.
38. Baran A, AtılganLülecioğlu A, Gao L, Yazıcı YY, Demirel F, Metin A, et al. A Novel Heterozygous NFKB2 Variant in a Multiplex Family with Common Variable Immune Deficiency and Autoantibodies Against Type I IFNs. J Clin Immunol. 2024;45(1):48.
39. Abraham RS, Marshall JM, Kuehn HS, Rueda CM, Gibbs A, Guider W, et al. Severe SARS-CoV-2 disease in the context of a NF-κB2 loss-of-function pathogenic variant. J Allergy Clin Immunol. 2021;147(2):532-544.e1.
40 Kwiatkowski V, Thanaputkaiporn N, Poowuttikul P. NFKB2 Mutation in 2 Siblings with DAVID Syndrome and CVID with Variable Presentations. J Allergy Clin Immunol. 2022;149(2):AB27.
41. Ospina FE, Echeverri A, Zambrano D, Suso JP, Martínez-Blanco J, Cañas CA, et al. Distinguishing infections vs flares in patients with systemic lupus erythematosus. Rheumatol Oxf Engl. 2017;56(suppl_1):i46-54.
42. Özdemir B, Erden A, Güven SC, Armagan B, Apaydin H, Karakas Ö, et al. COVID-19 and eosinophilic granulomatosis with polyangiitis or COVID-19 mimicking eosinophilic granulomatosis with polyangiitis? Rheumatol Int. 2021;41(8):1515–21.
43. Hellmich B, Sanchez-Alamo B, Schirmer JH, Berti A, Blockmans D, Cid MC, et al. EULAR recommendations for the management of ANCA-associated vasculitis: 2022 update. Ann Rheum Dis. 2024;83(1):30–47.
44. Chung SA, Langford CA, Maz M, Abril A, Gorelik M, Guyatt G, et al. 2021 American College of Rheumatology/Vasculitis Foundation Guideline for the Management of Antineutrophil Cytoplasmic Antibody-Associated Vasculitis. Arthritis Care Res. 2021;73(8):1088–105.

45. Peng JM, Du B, Qin HY, Wang Q, Shi Y. Metagenomic next-generation sequencing for the diagnosis of suspected pneumonia in immunocompromised patients. J Infect. 2021;82(4):22–7.
46. Deng W, Xu H, Wu Y, Li J. Diagnostic value of bronchoalveolar lavage fluid metagenomic next-generation sequencing in pediatric pneumonia. Front Cell Infect Microbiol. 2022;27(12):950531.

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