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Case Report

Diagnosis by metagenomic next-generation sequencing of invasive pulmonary aspergillosis in an infant with chronic granulomatous disease

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ABSTRACT

Invasive pulmonary aspergillosis (IPA) is a serious fungal infection, with a high degree of mortality in immunocompromised individuals. Diagnosis of IPA is challenging in that clinical manifestations are not specific, with sensitivity of traditional detection procedures low. We report a case of IPA in a chronic granulomatous disease (CGD) infant who was initially suspected to have a lung tumor. *Aspergillus fumigatus* was identified as the pathogen in bronchoalveolar lavage fluid (BALF) by next-generation sequencing (mNGS). The patient recovered rapidly following a change of appropriate antifungal treatment and was discharged. This case highlights the additional value of BALF-mNGS for the diagnosis of pediatric invasive pulmonary fungal infection in immunedeficient children.

1. Introduction

Invasive pulmonary aspergillosis (IPA) is a life-threatening lung disease of immunocompromised individuals. IPA is typically caused by Aspergillus fumigatus, a species of fungus with a tendency for infection of pulmonary blood vessels and bronchi [1]. As a well-known complication, IPA is a leading cause of death for patients with chronic granulomatous disease (CGD), a primary immunodeficiency disorder that increases a patient's susceptibility to severe infections caused by particular bacteria and fungi [2]. Thus, it is very important to identify the pathogen and begin pathogen-directed therapy as soon as possible for CGD patients complicated by IPA. In this study, we report a case of IPA in an infant with CGD in which the pathogen was quickly detected by bronchoalveolar lavage fluid (BALF) next-generation sequencing (mNGS) and treated with appropriate antifungal therapy. This report uses mNGS to clarify the etiology of IPA. Case details and clinical characteristics of IPA are summarized in order to improve disease outcomes and to reduce misdiagnosis.

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2. Case description

The patient was a 2-month and 18-day old boy, who had experienced shortness of breath and feeding pause since birth. At the age of one and a half months, the shortness of breath became more obvious and the infant was cyanotic when feeding. The frequency of feeding pause and cough also increased, but no fever was noted. Nine days before admission, the patient was examined by chest computed tomography (CT) at a local hospital, with neoplastic lesions suspected in the right lung. Initially, the patient received oxygen therapy, amoxicillin-potassium clavulanate, and gamma globulin injection (400 mg/kg) because of an increased white blood cell (WBC) and C-reactive protein level (CRP). Four days later the patient was transferred to another hospital where he was treated with oxygen therapy and cefoperazone/sulbactam for 3 days. These treatments were not effective and the patient continued to experience shortness of breath and fever. He was transferred to our hospital with vital signs of T 38 °C, P 172 beats/min, and R 42 breaths/min, as well as shortness of breath and signs of increased inspiratory load (e.g. three concave sign positive). As the condition progressed to respiratory distress and carbon dioxide retention, the infant was admitted to the pediatric intensive care unit (PICU) and received noninvasive ventilation immediately.

For routine blood tests performed in other hospitals, all WBC counts were found to exceed the normal range, with a minimal value of 15.26 × 10^9/L and a maximal value of 34.1 × 10^9/L. In our hospital, WBC ranged from 17.31 × 10^9/L to 26.63 × 10^9/L. The concentrations of CRP and procalcitonin (PCT) upon admission were 34.99 mg/L and 0.21 ng/mL. Similarly, the (1, 3)-β-D-glucan test (G test) initially showed a significantly high value of 177.2 pg/mL (cutoff value is 100 pg/mL). Initially, no pathogen was found in the sputum (by smear microscopy), blood, sputum, or BALF cultures. Other tests were also normal including immunoglobulin level, lymphocyte count, tuberculosis infection test (T-SPOT.TB), and cytomegalovirus IgM antibody levels.

In the absence of evidence of pathogens, we can only choose empirical treatment. Despite this, the child was still short of breath and febrile. CT scans taken before treatment (Fig. 1A and B) showed inflammatory lesions in both lungs, along with a mass and incomplete expansion of the upper right lung. CT-guided lung puncture pathological examination was performed because of a suspected tumor. Pathological examination revealed granulomatous lesions in right lung tissue with a nodular structure and an infiltration of a large number of lymphocytes, plasma cells, neutrophils, and scattered multinucleated giant cells. Gram stain, AFB stain, Fite stain, and Silver stain excluded neoplastic lesions. Therefore, we performed fiberoptic bronchoscopy and BALF-mNGS examination. The location of the lesion was determined by chest imaging examination, and the most significant part of the lesion was selected for bronchoalveolar lavage. BALF of the child was collected according to Chinese Guidelines for Pediatric Flexible Bronchoscopy, the total "recovery time" should be > 30%, and the "lavage time" should be controlled within 5 minutes [3]. Nucleic acid was extracted from 5 mL of BALF followed by library construction and high-throughput sequencing performed with an Illumina Nextseq 550Dx sequencer. By sequence alignment, BALF mNGS identified *A. fumigatus* on day 10 (Fig. 2A) and day 28 (Fig. 2B). Prior to this, antibiotic treatment was ineffective. Combined with clinical manifestations, lung CT scan, lung puncture pathology, elevated G test value, all of which made us suspect a fungal infection. After Aspergillus was found, we immediately changed to targeted therapy with intravenous

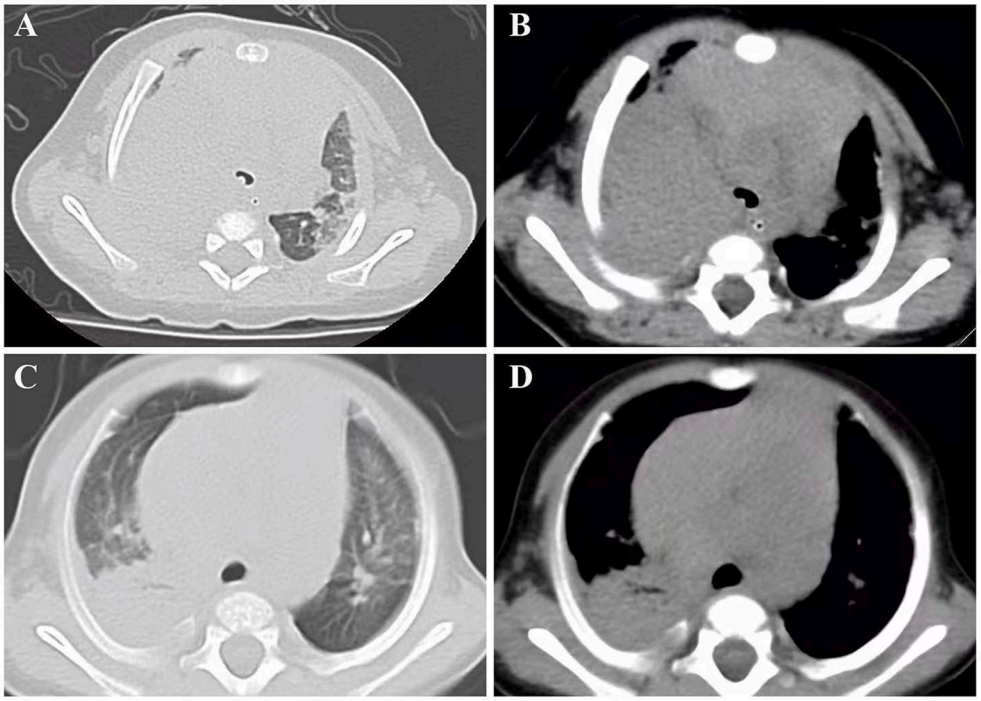

Fig. 1. CT scans before treatment (A, B) and 3 months later (C, D).

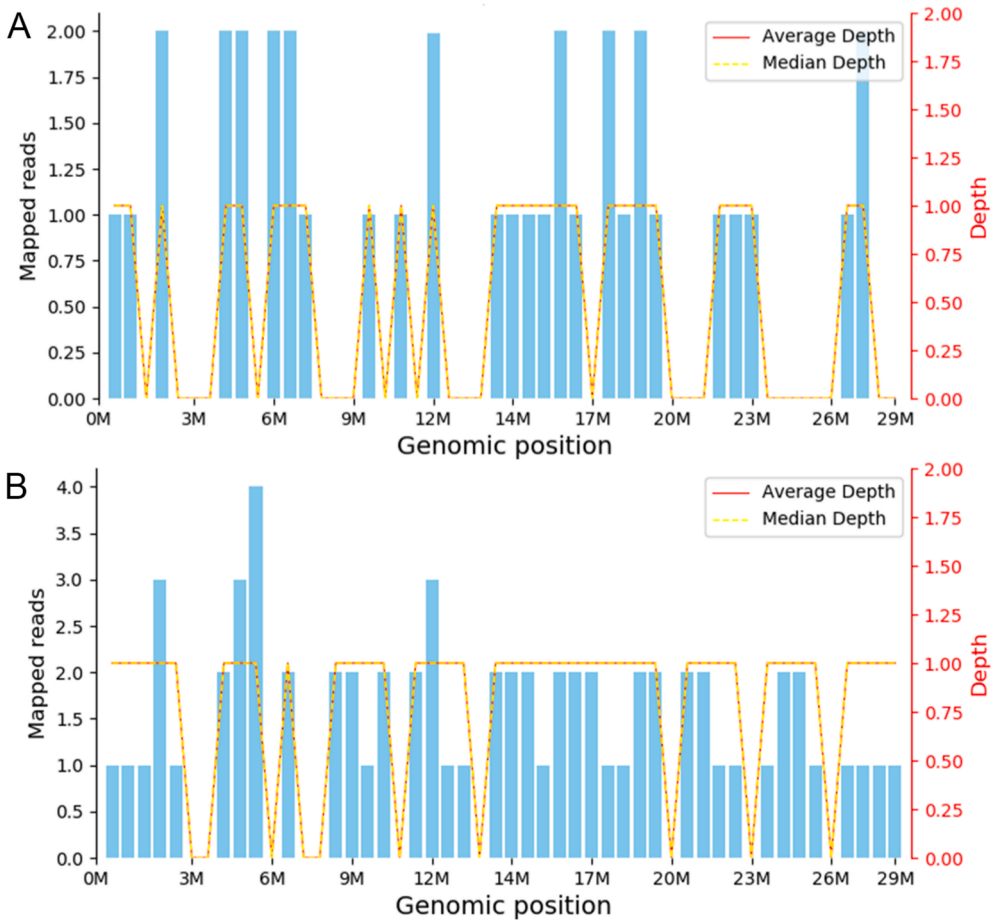

Fig. 2. (A) Eleven days after admission, diagram shows 23 *A. fumigatus* reads. (B) Twenty-eight days after admission, diagram shows 64 *A. fumigatus* reads.

caspofungin, and the condition gradually improved, body temperature returned to normal and breathing improved. Two weeks later, it was changed to sequential oral therapy of voriconazole. The treatment process is outlined in (Fig. 3).

Aspergillus is a common pathogen in people with immunodeficiency, so we have also improved the genetic testing. We found compound heterozygous mutations in the neutrophil cytosolic factor 1 gene (NCF1), consisting of a heterozygous mutation of paternal NCF1, and a deletion mutation of the maternal NCF1 that resulted in a large deletion of chromosomal segments (copy-number variations) of the gene (Fig. 4). The patient was diagnosed with autosomal recessive cytochrome b-positive CGD type I [4].

The patient had oral voriconazole sequential therapy after discharge and remained in good condition through 3-months of follow-up. The CT scans showed inflammatory lesions in both lungs and incomplete expansion of the upper right lung, with significantly fewer lesions (Fig. 1C and D), without any symptoms of fever or shortness of breath.

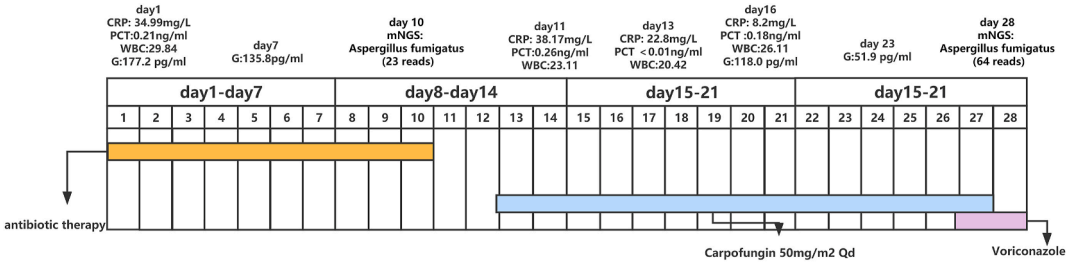

Fig. 3. Details of inpatient treatment.

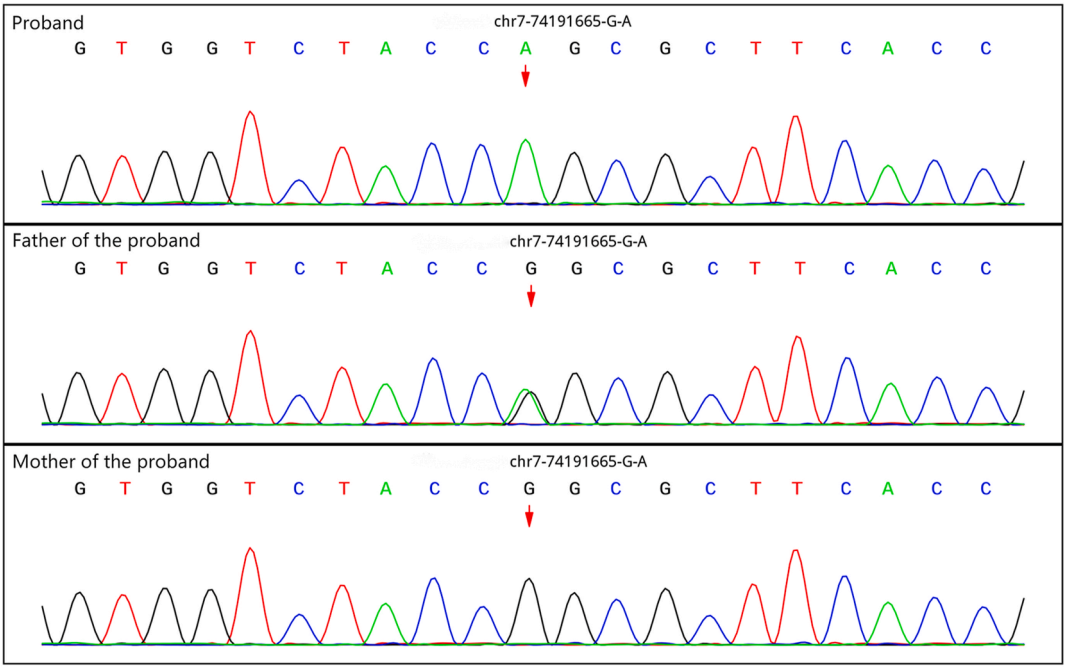

Fig. 4. Sequencing maps of NCF1 variants in the infant and his parents.

3. Discussion

Invasive aspergillosis is a severe fungal infection that primarily affects people with a weakened immune system [5]. This infection typically manifests with severe lung involvement, a poor prognosis, and a high mortality rate approaching 50% [6]. A study from Taiwan reported a high mortality rate (30.22%) for IPA cases assessed from 2002 to 2012 [7]. Unfortunately, diagnosis of IPA is difficult. To improve prognosis and reduce IPA mortality, it is essential to minimize effects of the pathogen by timely and rapid diagnosis, followed by appropriate treatment.

Diagnosis of Aspergillus infections is a challenge in that specific clinical manifestations are lacking. The traditional 'gold standard' is microbial culture, but Aspergillus culture requires an extended time period and has a low detection sensitivity. Calitri et al. [8] suggested the G test as a screening tool for early diagnosis of invasive fungal disease in children, but this suggestion has not been widely accepted because of many false-positives including piperacillin treatment, hemodialysis, blood product transfusion, and bacteremia [9]. In immunosuppressed and critically ill patients, the G test has a sensitivity of 88% and a specificity of 82% for probable IPA [10]. Galactomannan is a polysaccharide present in the cell wall of Aspergillus. Galactomannan enzyme immunoassay (GM-EIA) has good sensitivity and specificity for early detection of invasive aspergillosis. Gefen et al. [11] reported 80% sensitivity and 66% specificity, with false positivity rates range from 10% to 44% among pediatric patients [12]. Further, a variety of exposure factors have been reported to produce false-positive results for this test such as age, existing disease, infectious bacteria antibiotics, nutritional support, and even food taken during the hospitalization, which limit its utility [13,14]. Currently, CT is the radiologic cornerstone procedure for diagnosis of invasive pulmonary fungal infection, even though CT characteristics are not specific for IPA. A multicenter retrospective analysis of IPA concluded that the most common radiologic feature was lung nodules (34.6%), with most children exhibiting; halo sign (11%, an early sign of IPA), cavitation (24.5%), or air crescent sign (2.2%), each of which prompt diagnosis [15–17]. Histopathology is often recommended as a further diagnostic support for an IPA diagnosis. However, a sufficient quantity of the desired biopsy sample is difficult to obtain from an infected site, resulting in a relatively high false-negative rate. Further, biopsy samples do not reflect the extent of the infected area and are insufficient when used alone.

In order to improve the accuracy of IPA diagnosis and to justify IPA treatment, it is necessary to utilize all possible diagnostic methodologies including conventional morphological examination, various culture- and non-culture-based methods, as well as other adjunctive laboratory tests, together with a variety of emerging diagnostic techniques. In this case, results of blood, sputum, and BALF culture were negative, and other tests cannot identify the pathogen, providing no other alternative than empirical antibiotic therapy. Therefore, we should adopt more effective diagnostic methods, such as BALF-mNGS used in this report. We extracted nucleic acid from 5 mL of BALF, completed high-throughput sequencing, and identified *A. fumigatus.* CT scan showed a focal mass. Lung histopathology suggested inflammatory granulomatosis. With an elevated G test, IPA was considered as the clinical diagnosis. Considering the child's age and critical condition, we suspected an immune deficiency. Based on the literature [18,19],intravenous caspofungin was administered for 2-weeks and the patient's conditions improved dramatically. At discharge, oral voriconazole was prescribed for subsequent therapy.

Unbiased mNGS is a promising and innovative tool for pathogen detection, by which almost all nucleic acids (DNA and/or RNA) of a specimen can be sequenced in parallel. mNGS is a hypothesis-free diagnostic platform that allows for direct pathogen detection from

a clinical specimen without primers or probes, making it extremely useful for diagnosis of unknown infections. In the case of fungi, genomes of more than one thousand species are publicly available, substantially providing for faster and more accurate diagnostic analysis of pathogenic strains [20]. Nonetheless, the mNGS method does have a few restrictions including colonization differentiation, infection and contamination, interference of human nucleotide sequences (DNA), matched reads, and cell-free DNA extraction [21,22]. Currently, even if we can identify the causative organism by mNGS, it is not possible to know the drug susceptibility of the organism. Further, mNGS method has not yet been established as an indicator of therapeutic efficacy.

Retrospective studies comparing the performance of mNGS and traditional culture methodology demonstrated mNGS to have a higher sensitivity (50.7%) and specificity (85.7%), with sensitivity statistically different based on whether the samples was blood, BALF, or sputum [23,24]. We found BALF to be the most suitable, yielding a higher load than sputum or blood, with sensitivity and specificity for BALF-mNGS of 84.4% and 85.3%, respectively, which is quite suitable for the diagnosis of pulmonary fungal infection [25,26]. For suspected *Aspergillus* infections, the BALF-mNGS detection rate is high and is more suitable than other respiratory samples [27,28]. In this case, we could only initially provide empirical treatment based on the patient's medical history, clinical manifestations, and examination results. The importance of mNGS what that anti-fungal targeted therapy was administered and the patient recovered.

Thus, mNGS is an alternative diagnostic method that can be used to assist clinicians in their decision-making process. This case report validates the feasibility of direct pathogen identification from BALF by short-read whole-genome sequencing, with a mNGS platform. This approach is especially valuable for immunocompromised patients, such as the infant with CGD reported in this case. When combined with modern imaging technology and other adjunctive laboratory tests, mNGS-based pathogen identification has great potential for acceleration of the diagnosis process, increasing the pathogen detection rate, and allowing for timely initiation of appropriate antimycotics, which limits unnecessary side effects and improves patient outcomes.

4. Conclusions

mNGS is a powerful tool that allows for direct simultaneous detection of almost all species within a clinical sample. This case report demonstrates mNGS to be an auxiliary tool for the diagnosis of pulmonary fungal infections. As a new diagnostic technology, mNGS is an excellent means by which to identify pathogens. These preliminary finding should be confirmed by future diagnostic clinical trials.

Informed consent statement

Written informed consent was obtained from the patient to publish this paper.

Ethics approval and consent to participate

This study is in accordance with the guidelines for human research and the Declaration of Helsinki. This research was approved by the medical ethics committee of the Guangdong Provincial People's Hospital, China (KY-Q-2021-260-01).

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None.

Author contributions

AY, YG and GZ made substantial contributions to the conception and design of the study, as well as critical revision of the manuscript with regard to important intellectual content. AY wrote the first draft of the manuscript, and CW wrote portions of the manuscript. JW and YS performed bronchoscopy and collected alveolar lavage fluid. PC participate in data collection and collation. JZ completed the follow-up. ZZ provided imaging data, JL provided pathology data, and JY provided mNGS data. All authors revised, read, and reviewed the final manuscript.

Availability of data and materials

The datasets generated and analyzed during the current study are available in the NCBI BioProject database under accession number PRJNA849374 (https://www.ncbi.nlm.nih.gov/bioproject),

Declaration of competing interest

The authors have no conflicts of interest to declare.

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None.

Abbreviations

IPA	Invasive pulmonary aspergillosis
mNGS	Metagenomic next-generation sequencing
BALF	Bronchoalveolar lavage fluid
WBC	White blood cell
CRP	C-reactive protein
CT	Computed tomography
CGD	Chronic granulomatous disease
G test	(1, 3)-β-D-glucan test

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