

# Disseminated *Nannizziopsis* Infection in an Adolescent With a *STAT1* Mutation

Zachary M. Most,<sup>1,6</sup> Tiffany Lieu,<sup>2</sup> Laura Filkins,<sup>3</sup> Rory Nicolaidis,<sup>4</sup> Dinesh Rakheja,<sup>3</sup> Andrew Gelfand,<sup>5</sup> and Jeffrey Kahn<sup>1,6</sup>

<sup>1</sup>University of Texas Southwestern Medical Center, Department of Pediatrics, Division of Infectious Disease, Dallas, Texas, USA, <sup>2</sup>University of Texas Southwestern Medical Center, Department of Pediatrics, Division of Hospital Medicine, Dallas, Texas, USA, <sup>3</sup>University of Texas Southwestern Medical Center, Department of Pathology, Dallas, Texas, USA, <sup>4</sup>University of Texas Southwestern Medical Center, Department of Pediatrics, Division of Allergy and Immunology, Dallas, Texas, USA, <sup>5</sup>University of Texas Southwestern Medical Center, Department of Pediatrics, Division of Respiratory Medicine, Dallas, Texas, USA, and <sup>6</sup>University of Texas Southwestern Medical Center, Department of Microbiology, Dallas, Texas, USA

An adolescent with failure to thrive developed cuboid bone osteomyelitis and brain abscesses. Mold isolated from both locations was identified by universal genetic sequencing as *Nannizziopsis* spp, which is typically a pathogen of reptiles. The patient was subsequently diagnosed with a *STAT1* mutation and was successfully treated.

**Keywords.** fungal infections; immunocompromised host; *Nannizziopsis*; *STAT1*; voriconazole.

Many clinical laboratories rely on phenotypic characteristics for the identification of mold isolates, but this technique has several limitations. The library of potential molds that can be identified is relatively small; however, identification of the infecting organism can have implications for patients and should be sought.

In this case, we describe a patient with failure to thrive who had emigrated from West Africa to Texas and was diagnosed with an opportunistic disseminated mold infection. Deoxyribonucleic acid (DNA) sequencing was required to identify the organism to the genus level. This diagnosis confirmed that the patient had an unrecognized immunodeficiency despite extensive testing, which prompted further molecular investigation, and the patient was diagnosed with a gain-of-function (GOF) *STAT1* mutation. The signal transducer and activator of transcription (STAT) family of proteins regulate interferon-mediated immunity, and mutations in *STAT1* can increase susceptibility to various viral, mycobacterial, and fungal infections.

Received 3 July 2020; editorial decision 20 August 2020; accepted 24 August 2020.

Correspondence: Zachary M. Most, MD University of Texas Southwestern Department of Pediatrics, 5323 Harry Hines Blvd., Dallas, TX 75390 (zachary.most@utsouthwestern.edu).

## Open Forum Infectious Diseases®

© The Author(s) 2020. Published by Oxford University Press on behalf of Infectious Diseases Society of America. This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs licence (<http://creativecommons.org/licenses/by-nc-nd/4.0/>), which permits non-commercial reproduction and distribution of the work, in any medium, provided the original work is not altered or transformed in any way, and that the work is properly cited. For commercial re-use, please contact journals.permissions@oup.com  
DOI: 10.1093/ofid/ofaa390

## METHODS

### Fungal Identification by Universal Ribosomal Gene Sequencing and Phylogenetic Comparison

Fungal specimens collected from mold cultured in the laboratory and directly from a deep tissue biopsy were sent to molecular diagnosis clinical laboratories for identification by broad-range polymerase chain reaction and sequencing. Primer sets (designed by University of Washington Department of Laboratory Medicine) targeted highly conserved sequences flanking D1/D2 of the fungal 28S rRNA gene, internal transcribed spacer (ITS)1, and ITS2 regions [1]. Nucleic acid amplicon of the 3 target regions was Sanger sequenced, and a criterion of at least 99% nucleotide identity compared with database sequences was required for species-level identification and 97% nucleotide identity for genus-level identification. The ITS1 sequence from the brain lesion was used for phylogenetic comparison at our institution. Sequences were aligned [2] and compared using MEGA X (v 10.1.8) software for sequence alignment and evolutionary analysis, as described in Figure 2.

### Massively Parallel (Next Generation) Gene Sequencing Panel

A clinically validated Immunodeficiency Gene Sequencing panel (Children's Health Laboratory, Dallas, TX) was performed on the patient's extracted DNA using the Library Preparation Kit HTP (Kapa Biosystems). This panel includes the coding exons and flanking splice sites of 105 genes implicated in immunological disorders in children. Massively parallel sequencing was performed on the Illumina MiSeq instrument, and the University of Texas Southwestern BioHPC NGS-Pipeline v4.0 was used to perform alignment, variant calling, and data analysis. Variants predicted to be pathogenic or likely pathogenic were confirmed by Sanger sequencing on the ABI 3500xL Genetic Analyzer.

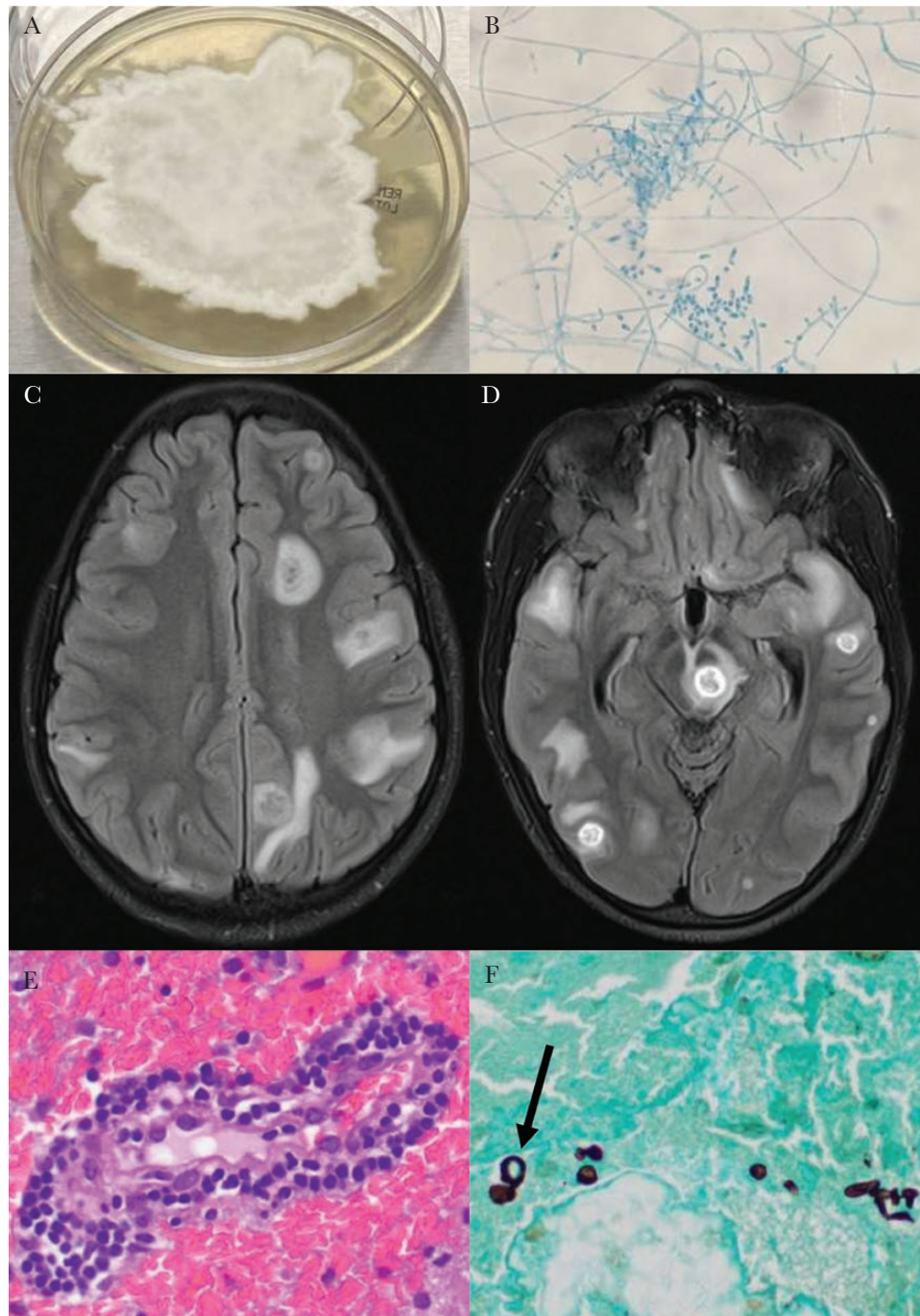
### Patient Consent Statement

The patient provided assent and her legal guardian provided written consent for this case report to be published. Per University of Texas Southwestern Institutional Review Board policy, a case report is not considered research and does not require institutional review board review. Health Insurance Portability and Accountability Act (HIPAA) privacy rules have been followed.

## RESULTS

### The Case

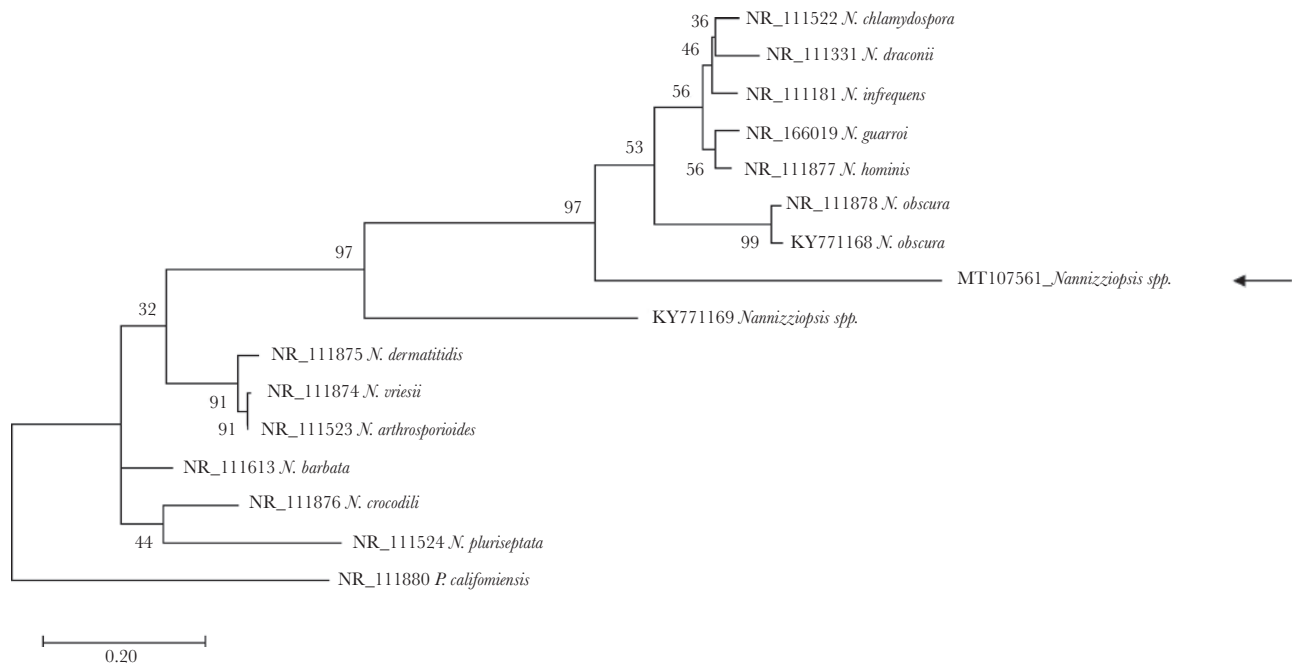
A 13-year-old girl with bronchiectasis and malnutrition was admitted due to worsening pulmonary function and 6 weeks of right ankle pain and swelling. She had a lifelong history of



**Figure 1.** (A) Photograph of the mold obtained from a right cuboid bone biopsy grown in culture on Sabouraud dextrose agar, showing a white cottony appearance after 4 days of agar medium growth from subculture. (B) Photomicrograph of the mold grown in culture from the same right cuboid bone biopsy (lactophenol cotton blue stain,  $\times 400$  magnification) demonstrating the architecture of the mold. (C and D) Pre- and postgadolinium (respectively) magnetic resonance images at separate axial slices on the first day of treatment. (E) Photomicrograph of a brain biopsy specimen (hematoxylin and eosin,  $\times 400$  original magnification) showing perivascular lymphocytic inflammation, hemorrhage, and gliosis. (F) Photomicrograph of the brain biopsy specimen (Grocott's methanamine silver stain,  $\times 400$  original magnification) showing few fungal hyphae (arrow).

chronic productive cough, severe malnutrition, and several episodes of thrush and aphthous stomatitis without a unifying diagnosis. Previous evaluations revealed bronchiectasis and a left lower lobe cavitory lesion. She had a normal sweat chloride test, normal ciliary structure on nasal brush biopsy,

and an evaluation for immunodeficiency had been unrevealing ([Supplementary Table S1](#)). Her forced expiratory volume in 1 second (FEV<sub>1</sub>) had decreased to 30% of predicted. Family history was remarkable for the death of her mother at age 34 due to breast cancer diagnosed 5 years earlier. Her mother also



**Figure 2.** Phylogenetic analysis of the internal transcribed spacer 1 (ITS1) DNA sequence obtained from the patient's brain tissue. Comparison nucleotide sequences were identified in GenBank using the National Center for Biotechnology Information Basic Local Alignment Search Tool (NCBI BLAST). Two of the nearest nucleotide match sequences along with 12 type sequences for different *Nannizziopsis* species and 1 type sequence from *Paranannizziopsis californiensis* were selected and aligned using MUSCLE [2] within the MEGA X (v10.1.8) software. A substitution model selection algorithm was used, and the Hasegawa-Kishino-Yano model with a discrete gamma distribution was selected based on having the lowest Bayesian information criterion. A rooted phylogenetic tree (with *P californiensis* as an outgroup) was created based on genetic distance calculated using a maximum-likelihood algorithm. The reliability of the tree was assessed with 1000 bootstrap replicates (the number at each branch point is the percentage of replicate trees in which the associated taxa are grouped together). The tree bars are drawn to scale, and the scale bar denotes the genetic distance in nucleotide base substitutions per site. The sequence from this case report is marked by an arrow.

suffered from chronic onychomycosis. This patient was born in Nigeria, and after her mother's death she moved to Texas to be raised by her maternal aunt.

At the time of admission, she was tachycardic and afebrile with otherwise normal vital signs. She weighed 32 kg (1st percentile) and was 152-cm tall (10th percentile). On examination, there were crackles in all lung fields and clubbing of her fingers and toes. She experienced tenderness to palpation over the lateral aspect of her right ankle. Magnetic resonance imaging (MRI) of her right foot revealed osteomyelitis of the cuboid bone with a calcaneocuboid joint effusion. On hospital day 9, she underwent incision with irrigation and drainage of her right calcaneocuboid joint and grossly purulent fluid was evacuated. Gram staining of the fluid identified few polymorphonuclear neutrophils and no microorganisms. Histopathologic examination showed acute osteomyelitis, and a periodic acid-Schiff stain highlighted few fungal hyphae (Supplementary Figure S1). Tissue from her cuboid bone sent for culture grew a mold with a cottony appearance with microscopically abundant arthroconidia and septate branching hyphae (Figure 1A and B). The mold could not be identified based on morphologic criteria; therefore, rDNA gene sequencing was performed. Meanwhile, empiric liposomal amphotericin B (5 mg per kg) was initiated on hospital day 12.

On hospital day 13, she developed severe, unrelenting headaches along with nausea, vomiting, bradycardia, and altered mental status. MRI of her brain with and without contrast showed numerous enhancing lesions with surrounding vasogenic edema throughout the brain including both hemispheres of the cerebrum and cerebellum and the left midbrain (Figure 1C and D). She developed hydrocephalus, so on hospital day 23 an intraventricular reservoir was placed and a brain biopsy specimen was obtained. Histopathologic examination showed perivascular lymphocytes, diffuse gliosis, and the presence of rare fungal hyphae (Figure 1E and F). Culture of the brain tissue was positive for the same mold. Voriconazole was empirically added for broader antifungal coverage and dexamethasone was given to reduce cerebral edema.

#### Genetic Analysis and Phenotypic Characterization of the Mold

Based on the nucleotide sequences, the mold from her bone and brain were identified as *Nannizziopsis* species. Species-level identification was not met by any sample (Supplementary Figure S2). The ITS1 region contained a 130-base pair insertion that was not found in any *Nannizziopsis* spp database sequences (Supplementary Figure S3). Phylogenetic analysis of the ITS1 region further suggests that this patient's strain of *Nannizziopsis* is not closely related to other reported strains (Figure 2). The

**Table 1. *Nannizziopsis* Infections in Humans**

Year	Age/Sex	Species	Disease	Risk Factors	Treatment	Outcome	Reference
2018	13/F	Unk	Disseminated, osteomyelitis, pneumonia, brain abscesses	<i>STAT1</i> GOF mutation, born in Nigeria, snake skin consumption	Amphotericin B and voriconazole	Recovery	This report
2017	52/F	Unk	Brain abscesses	HIV, living in Mali	Amphotericin B and voriconazole	Recovery with neurologic sequelae	Nourrisson et al [3]
2015	63/F	<i>Nannizziopsis obscura</i>	Brain abscesses	Leukemia, travel to Senegal	None	Death	Nourrisson et al [3]
2015	34/M	<i>N. obscura</i>	Disseminated, rash, thoracic paraspinal abscess, lymphadenopathy	Kidney transplant, frequent travel to the Gambia	Posaconazole	Recovery	Baggott et al [4]
2005	38/M	<i>N. obscura</i>	Disseminated, brain abscesses, lung nodules	HIV, born in Nigeria	Voriconazole	Recovery	Steininger et al [6]
2004	40/M	<i>Nannizziopsis infrequens</i>	Pneumonia vs contaminant	HIV	None	Recovery	Brandt et al [7]
2000	32/M	<i>Nannizziopsis hominis</i>	Disseminated, lymphadenopathy, endocarditis,	Travel to Nigeria	Itraconazole	Unk	Stchigel et al [9]
1994	Unk/M	<i>N. hominis</i>	Deep muscle mass	HIV/AIDS	Itraconazole	Death	Sigler et al [5]
1982	24/M	<i>N. obscura</i>	Osteomyelitis	Travel to Africa	Amphotericin B	Recovery	Stillwell et al [8]

Abbreviations: AIDS, acquired immunodeficiency syndrome; GOF, gain-of-function; HIV, human immunodeficiency virus; *STAT1*, signal transducer and activator of transcription 1; Unk, unknown or missing information.

strain from her cuboid bone had a voriconazole minimum inhibitory concentration (MIC) of 0.25 µg/mL (Supplementary Table S2).

#### Immunodeficiency Evaluation

An enumeration assay revealed a decreased percentage of T-helper 17 cells (0.13%, adult reference range 0.31%–1.80%), indicating a level of immune dysfunction. The next-generation gene sequencing panel revealed a heterozygous missense variant in exon 14 of the *STAT1* gene located on short arm of chromosome 2 (*STAT1*:NM\_007315.3:c.1154C>T het p.(Thr385Met)).

#### Antifungal Therapy and Outcome

Amphotericin B was discontinued once her voriconazole levels were therapeutic. Over the next week her headache and foot pain resolved and her appetite improved. On hospital day 49 she was discharged home on oral voriconazole. By her 4-month follow-up, she had no headache, repeat brain imaging showed near resolution of her brain lesions, and her FEV<sub>1</sub> improved to 64% of predicted. Eleven months after her admission her intraventricular reservoir was removed. Upon further questioning, her aunt revealed that shortly after the patient was born, her mother fed her crushed snake skin powder by mouth as a folk remedy. There was no other reported direct contact with reptiles.

#### DISCUSSION

Human infections with *Nannizziopsis* species are rare with only 8 cases reported in the medical literature (Table 1) [3–9].

Almost all reported cases have been in immunocompromised individuals who have lived in or traveled to West Africa, yet no specific source of infection or epidemiologic association has been identified. In humans, sites of infection have included bone and joint [8], skin [4], lungs [4, 7], brain [3, 6], and disseminated disease [4–6, 9]. Outcomes have ranged from full recovery with or without antifungal treatment to death, yet the optimal antifungal therapy and duration is unknown. Previous studies have reported low MICs to triazoles and amphotericin B, and patients have had successful therapy with oral triazoles. It is worth noting that reptiles are typically treated with triazoles [5].

This is the first reported case of an adolescent with a *Nannizziopsis* infection and the first to report a history specific for reptile skin exposure. It is unclear exactly how long she was infected with this mold, although she likely had subclinical or occult infection for several years, dating back to her time in Nigeria. She will remain on voriconazole at suppressive dosing (goal trough concentration between 1 and 2 µg/mL) to prevent recurrence of infection.

Mutations in *STAT1* exert effects primarily through impairment or enhancement of interferon-gamma and interferon-alpha/beta-mediated immunity, increasing host susceptibility to various viral, mycobacterial, and fungal infections [10]. This patient's specific variant is associated with GOF and was predicted to be likely pathogenic based on prior reports of association with a progressive combined immunodeficiency [11, 12] and chronic mucocutaneous candidiasis [13]. Because this patient's spectrum of susceptibility to infection

was unclear, intravenous immune globulin, azithromycin, and trimethoprim-sulfamethoxazole were initiated, in addition to voriconazole, for antimicrobial prophylaxis. This gene is inherited in an autosomal dominant manner, which suggests that her mother, who had chronic onychomycosis, may have also had this immunodeficiency. Given the wide spectrum of possible mutations and broad infection susceptibility phenotype, the importance of identifying genetic defects in patients with suspected immunodeficiency cannot be underestimated.

## CONCLUSIONS

In conclusion, we have identified a GOF-*STAT1* mutation in an adolescent who had a disseminated *Nannizziopsis* infection. This case highlights the power of genetic sequence analysis of both the host and the pathogen and provides additional insights into the role of the STAT family of proteins in the innate immune response to both common and uncommon pathogens.

## Supplementary Data

Supplementary materials are available at Open Forum Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

## Acknowledgments

We thank Dr. Christian Wysocki (University of Texas Southwestern Medical Center) for his clinical evaluation that ultimately led to this patient's diagnosis of immunodeficiency and for continuing her outpatient management.

**Potential conflicts of interest.** All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

## References

1. Wang XC, Liu C, Huang L, et al. ITS1: a DNA barcode better than ITS2 in eukaryotes? *Mol Ecol Resour* **2015**; 15:573–86.
2. Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* **2004**; 32:1792–7.
3. Nourrisson C, Vidal-Roux M, Cayot S, et al. Invasive infections caused by *Nannizziopsis* spp. molds in immunocompromised patients. *Emerg Infect Dis* **2018**; 24:549–52.
4. Baggott A, McGann H, Barton R, Ratner J. Disseminated *Nannizziopsis obscura* infection in a renal transplant patient- The first reported case. *Med Mycol Case Rep* **2017**; 17:20–4.
5. Sigler L, Hambleton S, Paré JA. Molecular characterization of reptile pathogens currently known as members of the chrysosporium anamorph of *Nannizziopsis vriesii* complex and relationship with some human-associated isolates. *J Clin Microbiol* **2013**; 51:3338–57.
6. Steining C, van Lunzen J, Sobottka I, et al. Mycotic brain abscess caused by opportunistic reptile pathogen. *Emerg Infect Dis* **2005**; 11:349–50.
7. Brandt ME, Gaunt D, Iqbal N, et al. False-positive *Histoplasma capsulatum* Gen-Probe chemiluminescent test result caused by a *Chrysosporium* species. *J Clin Microbiol* **2005**; 43:1456–8.
8. Stillwell WT, Rubin BD, Axelrod JL. *Chrysosporium*, a new causative agent in osteomyelitis. A case report. *Clin Orthop Relat Res* **1984**; 184:190–2.
9. Stchigel AM, Sutton DA, Cano-Lira JF, et al. Phylogeny of chrysosporidia infecting reptiles: proposal of the new family *Nannizziopsiaceae* and five new species. *Persoonia* **2013**; 31:86–100.
10. Sampaio EP, Hsu AP, Pechacek J, et al. Signal transducer and activator of transcription 1 (*STAT1*) gain-of-function mutations and disseminated coccidioidomycosis and histoplasmosis. *J Allergy Clin Immunol* **2013**; 131:1624–34.
11. Eslami N, Tavakol M, Mesdaghi M, et al. A gain-of-function mutation of *STAT1*: a novel genetic factor contributing to chronic mucocutaneous candidiasis. *Acta Microbiol Immunol Hung* **2017**; 64:191–201.
12. Sharfe N, Nahum A, Newell A, et al. Fatal combined immunodeficiency associated with heterozygous mutation in *STAT1*. *J Allergy Clin Immunol* **2014**; 133:807–17.
13. Depner M, Fuchs S, Raabe J, et al. The extended clinical phenotype of 26 patients with chronic mucocutaneous candidiasis due to gain-of-function mutations in *STAT1*. *J Clin Immunol* **2016**; 36:73–84.