Rapid and precise diagnosis of *T. marneffei* pulmonary infection in a HIV-negative patient with autosomal-dominant *STAT3* mutation: a case report

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Abstract

Background: *Talaromyces marneffei*, also named *Penicillium marneffei*, is an opportunistic pathogen that can cause systemic or limited infection in human beings. This infection is especially common in human immunodeficiency virus (HIV)-infected hosts; however, it has also been recently reported in HIV-negative hosts. Here, we report a very rarely seen case of *T. marneffei* pulmonary infection in a non-HIV-infected patient with *signal transducer and activator of transcription 3* (*STAT3*) mutation.

Case presentation: A 34-year-old woman was admitted to our hospital for uncontrollable nonproductive cough and dyspnea with exercise. She had been immunocompromised since infancy. Computerized tomography scan showed multiple ground glass opacities with multiple bullae in both lungs. Next generation sequencing (NGS) of the bronchoalveolar lavage fluid identified *T. marneffei* nucleotide sequences. Culture of bronchoscopy specimens further verified the results. The patient was HIV negative, and blood gene detection indicated *STAT3* mutation. To date, following the application of itraconazole, the patient has recovered satisfactorily.

Conclusion: In clinical practice, *T. marneffei* infection among HIV-negative individuals is relatively rare, and we found that patients who are congenitally immunocompromised due to *STAT3* mutation may be potential hosts. Early diagnosis and timely treatment are expected to improve the prognosis of *T. marneffei* infection. NGS is a powerful technique that may play an important role in this progress.

The reviews of this paper are available via the supplemental material section.

Keywords: human immunodeficiency virus (HIV)-negative, next generation sequencing, *signal transducer and activator of transcription 3* mutation, *Talaromyces marneffei*

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Background

Talaromyces marneffei, formerly called *Penicillium marneffei*, causes mostly opportunistic infections in immunodeficiency individuals, who are particularly susceptible to *T. marneffei*, especially human immunodeficiency virus (HIV)-positive patients in certain endemic regions such as Southeast Asia.[1] In 1973, the first case of *T. marneffei* infection was reported in an American minister in Southeast Asia.[2] The incidence rate of *T. marneffei* infection increased noticeably after the acquired immune deficiency syndrome (AIDS) pandemic in the 1980s.[1] Infection by *T. marneffei* is rarely reported in non-HIV-infected hosts,[3] but in recent years the incidence rate of *T. marneffei* infection in HIV-negative individuals is increasing year by year. Many of the HIV-negative non-endemic patients had potentially immunocompromising conditions, such as autosomal dominant hyper-IgE syndrome (AD-HIES), hyper-IgM syndrome,

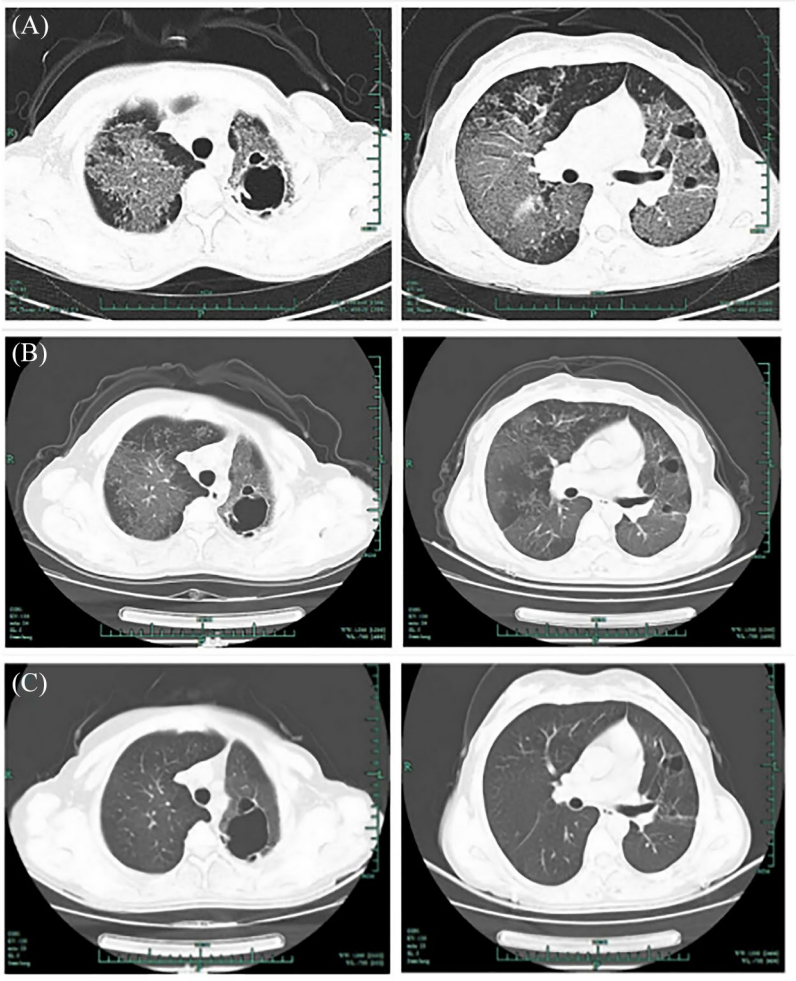

Figure 1. (A) Past chest CT scan (1 January 2019) showing multiple disseminated ground glass opacities with multiple bullae. (B, C) Chest CT scan during follow up (26 January 2019 and 27 April 2019, respectively) showing distinct resolution of both lungs.
CT, computed tomography.

immunosuppressive therapies, and being positive for anti-interferon-gamma autoantibody. Therefore, it is important to increase the diagnostic efficiency of this disease, especially in HIV-negative hosts, with a effective technique. Here, we report a rare case of a HIV-negative patient with lung *T. marneffei* infection with a *STAT3* (*signal transducer and activator of transcription 3*) mutation.

Case presentation

A 34-year-old young woman was admitted to our department for "recurrent cough for 6 months, acute exacerbation with dyspnea for 1 month" on 7 January 2019. The female presented with a 6-month history of slight nonproductive cough, shortness of breath after exercise, and complained of mild fever and night sweating with yellow-brown sputum for several days but denied chest pain. After the application of antibiotics (the detail was not clear) in a local hospital, temperature declined to normal, but she still had cough and dyspnea with exercise. A chest computed tomography (CT) scan (1 January 2019, Figure 1A) showed multiple ground glass opacities with multiple bullae in both lungs. The patient had been slightly immunocompromised (the detail was not clear) since infancy, and had undergone several surgical treatments for suspected pleurisy and skin infection around the left ear. She had a history of viral hepatitis B, but was not on regular treatment, and no smoking or alcohol history. She was born in Gansu province, northwest of China, and moved to Hangzhou 10 years ago.

On physical examination, vital signs appeared normal, moist rales could be heard in both lungs, and there was no obvious cyanosis. Her HIV test was negative. The serum CA125 and NSE levels were 65.3 (reference 0–33 kU/l), 21.9 (reference 0–16.6 μg/l), respectively. The total T-lymphocyte count and CD4+ were 900 and 380 cells/μl, respectively. Hemoglobin was only 91 g/l. Blood gas analysis was normal. Serum immunoglobulin (Ig)E, IgG, IgA, and IgM were all normal. The plasma galactomannan antigen test and serum cryptococcal antigen agglutination test were normal. The sputum and blood cultures for both fungus and bacteria and sputum acid-fast bacillus test were negative. Other routine laboratory examinations were normal, such as the white blood count, C-reactive protein, glucose, aminotransferases, creatinine, vasculitis antibodies, and autoantibodies. The CT scan showed multiple disseminated ground glass opacities with multiple bullae in both lungs (Figure 1A), no pleural effusion and pleural thickening, and no swollen superficial lymph nodes throughout the body, and no abnormal echocardiography, abdominal B-ultrasonic, and brain CT were observed.

Bronchoscopic examinations revealed uneven local membrane surface (Figure 2A), hypoechoic areas in group 7, 4R, and 11Rs mediastinal lymph nodes through convex-probe endobronchial ultrasound (Figure 2B), and hypoechoic shadow in the dorsal segment of the right lower lobe (RLL) through radial-probe endobronchial ultrasound (Figure 2C). Cultures of the bronchoalveolar lavage fluid (BALF) for bacteria and acid-fast bacilli were all negative. The galactomannan test

Table 1. NGS of BALF identified 566 *T. marneffei* nucleotide sequences.

Type	Category	Species	Latin name	Total_reads_ percent	Total reads
F	Penicillium	*Talaromyces mameffei*	*Penicillium marneffei*	–	566

BALF, bronchoalveolar lavage fluid; NGS, next generation sequencing.

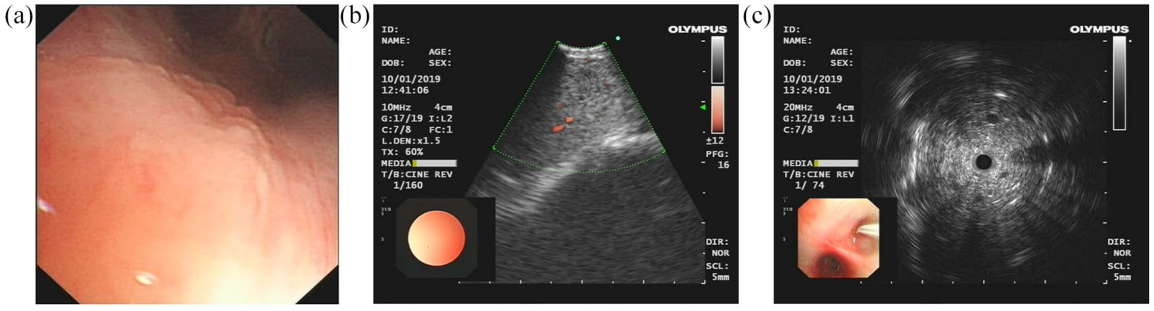

Figure 2. Bronchoscopy showed local uneven membrane surface (A), hypoechoic areas in group 7, 4R, and 11Rs mediastinal lymph nodes through CP-EBUS (B), and hypoechoic shadow in the dorsal segment of the right lower lobe through RP-EBUS (C).
CP-EBUS, convex-probe endobronchial ultrasound; RP-EBUS, radial-probe endobronchial ultrasound.

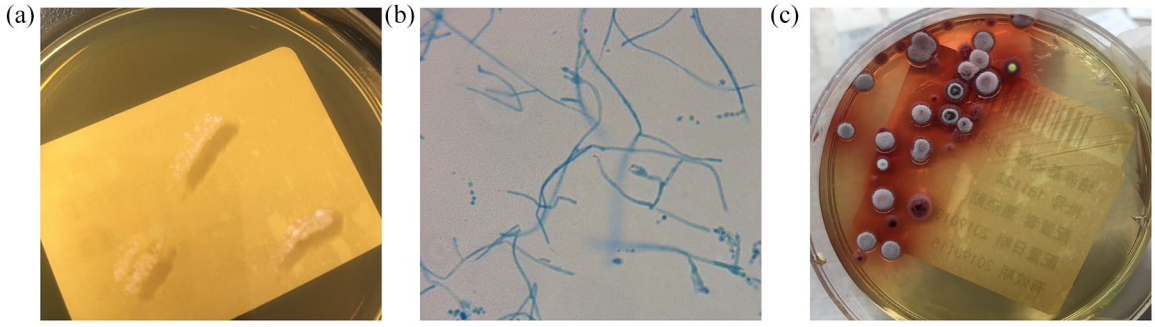

Figure 3. Culture of BALF revealed *Talaromyces marneffei*, which shows temperature-dependent dimorphic character, growing as yeast-like cells at 37°C (A) and as a mycelium at temperatures between 25°C and 30°C (B); the cells produced red pigment at 25°C (C).
BALF, bronchoalveolar lavage fluid.

and cryptococcal antigen agglutination test of BALF were also negative. Upon histological examination, chronic granulomatous inflammation was found in the dorsal segment of RLL and the group 7 lymph node. Next generation sequencing (NGS) of the BALF confirmed lung infection with *T. marneffei* 2 days later (Table 1). About 1 week later, culture of BALF and the biopsied tissue mass also showed the existence of *T. marneffei*. Based on the pathogen's temperature-dependent dimorphic growth characteristic and the production of soluble red pigment and PAS-negative cell content, the isolate was identified definitively as *T. marneffei* (Figure 3A–B).

On the other hand, considering that the patient's HIV test was negative but the total counts of lymphocytes and CD4+ were slightly decreased, together with her history since infancy, we could not rule out congenital immunodeficiency. A blood gene detection test was taken, which indicated a loss-of-function mutation in the gene *STAT3*; however, there were no similar mutations in her parents (Table 2, Figure 4A).

Table 2. Heterozygous missense mutation in exome regions of gene *STAT3* was identified by Sanger sequencing (c.92G>A, p.R31Q).

Patient and the family genetic detection					
Gene	**Inherit mode**	**Mutation information**	**Patient**	**Father**	**Mother**
STAT3	AD	C.92G>A chr17-40500443 p.R31Q	Heterozygous mutation	No mutation	No mutation
The detail gene detection result					
Gene	**Transcribed version exon number**	**Mutation ratio reference/ mutation**	**Hom/Het/Hem**	**gnomAD**	**ACMG**
STAT3	NM_139276.2 Exon2	49/45 (0.48)	Het	–	Likely pathogenic
Pathogenic	**Likely pathogenic**		**VUS**	**Likely benign**	**Benign**

STAT3, signal transducer and activator of transcription 3; AD, autosomal dominant; gnomAD, genome aggregation database; ACMG, American college of medical genetics and genomics.

So far, the patient was diagnosed as having a *STAT3* mutation and lung infection with *T. marneffei*. Considering her financial condition, she was prescribed oral itraconazole (200 mg, every 12 h) therapy on 13 January 2019 and later discharged. Two repeated chest CT, on 26 January 2019 (Figure 1B) and 27 April 2019 (Figure 1C), revealed distinct resolution of both lungs. The symptoms of shortness of breath and cough were also obviously alleviated, and the patient continues to receive treatment (itraconazole, 200 mg per day) and follow up at present.

Discussion and conclusion

This is a relatively rare case report of a *T. marneffei* pulmonary infection in a HIV-negative patient with *STAT3* mutation. The application of NGS in BALF greatly assists the rapid diagnosis of *T. marneffei* infection.

Patients with some immunodeficiencies, such as AIDS, AD-HIES, hyper-IgM syndrome, certain immunosuppressive therapies, and variety of transplants, are susceptible to *T. marneffei* as an opportunistic fungus.[4] Affected individuals often suffer quick progression to multiple organ failure and finally death. Infection by *T. marneffei* is rarely reported in non-HIV-infected hosts,[3] but in clinical practice, the incidence rate of *T. marneffei* infection in HIV-negative individuals is increasing year by year. In a report of five Chinese non-HIV-infected children and teenagers with *T. marneffei* infection, it was shown that four had had chronic mucocutaneous candidiasis since infancy, and one had AD-HIES.[5,6] Mutation in gene *STAT3* was identified by Sanger sequencing in our patient (c.92G>A, p.R31Q); however, neither parent carried a similar mutation (Figure 4A–C). *STAT3*, as a signal transducer and transcription factor, activates downstream of various of cytokine signals, including interferon-α, interleukin (IL)-6, and IL-10, and others.[7] It is reported that *STAT3* mutation is usually related to AD-HIES. AD-HIES is a very rare primary immunodeficiency, characterized by elevated serum IgE and eczema, recurrent skin infections, and sinopulmonary infections.[8] The classic triad of abscesses, pneumonia, and elevated IgE level was identified in 77% of all patients and 85% of those older than 8 years of age. At present, IgE is higher during childhood in some cases phenotypically, which may decrease, or even fall below normal, with age. The diagnostic criteria are not adequate for our patients. A consistent immunophenotype in AD-HIES patients is impaired development of Th17 lymphocytes, due mainly to the key role of cytokine signaling through *STAT3* in their generation. In addition, a loss-of-function mutation in the *STAT3* gene (STAT3-deficiency) is also frequently associated with susceptibility to fungal infections, including *Talaromyces* or aspergillosis,

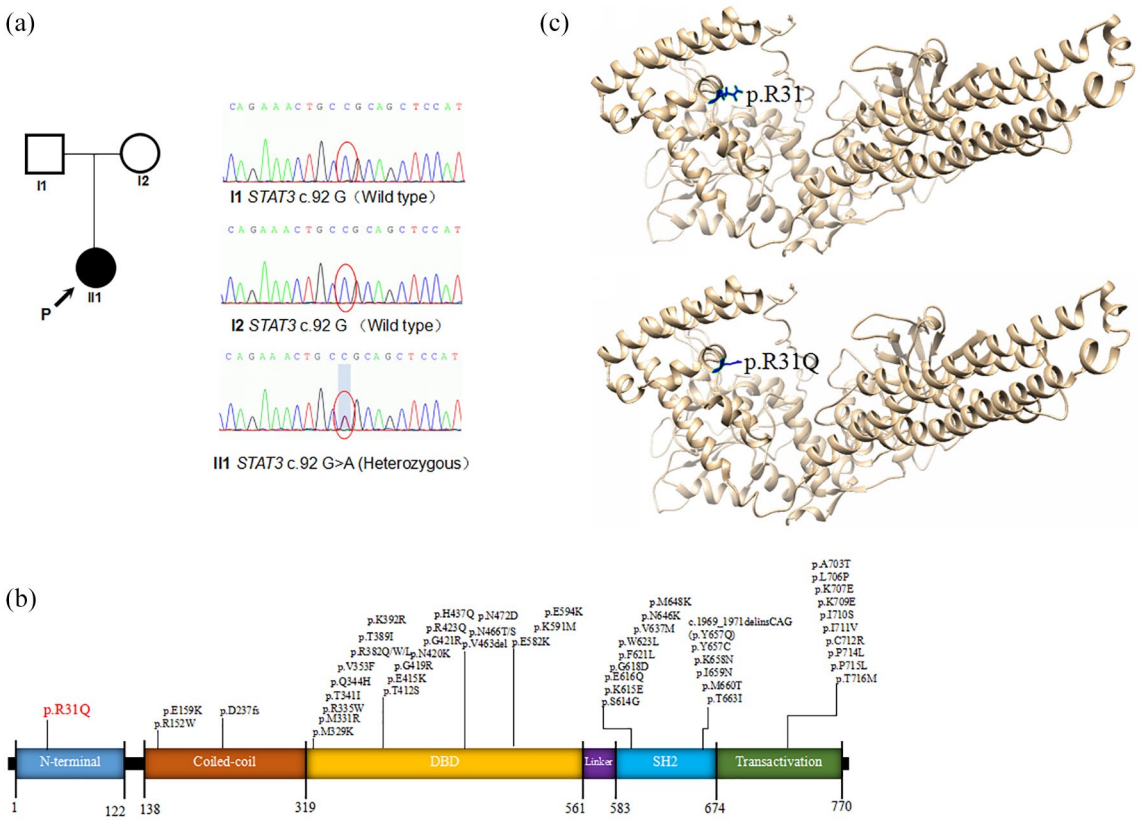

Figure 4. (A) Family map and Sanger sequencing. (B) Illustration of the functional structure of *STAT3*: p.R31Q in red and other known pathogenic, or likely pathogenic mutations, in black. (C) The labeling of p.R31Q on the structure of STAT3 modeled by the I-TASSER algorithm.
STAT3, signal transducer and activator of transcription 3.

although its pathogenesis remains largely unknown. STAT3-deficient patients showed a defective adaptive immune response, with lower production of cytokines, including IFN-γ, IL-17, and IL-22,[9] which could be the reason for their susceptibility to fungal infection in HIV-negative patients. Regrettably, cytokines were not detected and IgE is negative in this patient. From a clinical perspective, the patient fails to meet the recent diagnostic criteria of HIES. To conclude, a loss-of-function mutation in *STAT3* gene is a rare primary deficiency, and our study was limited in some aspects. We are not sure how big a role it plays in this case, but it indicates that STAT3-deficiency indeed increases susceptibility to microbial infections of lungs, which is the direction we are taking for our further research.

T. marneffei can lead to various kinds of infections involving multiple organs or systems, including the lungs, blood, skin, central nervous system, and bone marrow, so if not diagnosed or treated in a timely manner, it can be life-threatening. Whether in HIV-positive or HIV-negative patients, the clinical characteristics are similar.[10] The most common symptoms are cough, fever, anemia, weight loss, malaise, hepatosplenomegaly, and cutaneous lesions, but nonspecific and with little significance for differential diagnosis. The lung is the organ most commonly involved, occurring in 64% of HIV-infected patients and 75% of non-HIV-infected patients.[10] In this case, dyspnea was the main complaint along with recurrent cough; no other organs and systems seem to be involved.

Similarly in laboratory tests, there is no significant difference between HIV-negative and HIV-positive patients.[10] *T. marneffei* is well known to be the only temperature-dependent dimorphic pathogen in *Penicillium*, growing as a mycelium at temperatures 25°C–30°C with the generation of a soluble red pigment, and as yeast-like cells at 37°C. Only the yeast phase has pathogenicity. In addition, a

mulberry-shaped cell mass, sausage-shaped cells, and a transverse wall are the three main morphological characteristics of *T. marneffei* growth in tissue,[11] which were also found in our case.

In former clinical practice, the diagnosis of *T. marneffei* infection depended highly on tissue culture and histopathologic results, which can be confined to the low positive rate and can be time-consuming, respectively, especially as fungal cultures usually take about 3–7 days. We performed NGS on the patient's BALF, which detected numerous nucleotide sequence reads corresponding to *T. marneffei* 2 days later, and thus resulted in the timely diagnosis and treatment of *T. marneffei* pulmonary infection.

The successful application of NGS assisted the rapid diagnosis of *T. marneffei* infection, providing a powerful skill in clinical practice and revealing the potential value of this procedure in rapid etiological diagnosis.[12]

It is well known that amphotericin B, itraconazole, voriconazole, fluconazole, and terbinafine are the antifungal drugs most commonly used for therapy. In addition, itraconazole and amphotericin B are reported to be more effective in clinical practice, whereas the clinical response to fluconazole is relatively poor.[13] Current guidelines for the therapy of *T. marneffei* infection recommend amphotericin B treatment for 2 weeks, then adequate oral itraconazole for 10 weeks, and finally secondary prophylaxis.[14] From our case, we found that, following the application of oral itraconazole (200 mg, every 12 h) for 3 months, the patient recovered satisfactorily and lesions absorbed obviously on CT scans. To date, she continues to receive the application of itraconazole (200 mg per day) and follow up until the present. The general maintenance dose lasts for 1 year, depending on the efficacy or whether the risk factors can be terminated or not. However, despite standard treatment strategy, most infected individuals experience recurrence several months or even years later. Earlier research indicated that mortality in HIV-negative individuals was higher than in HIV-positive individuals,[10] which may be related partly to delayed diagnosis because of the lack of an effective and rapid diagnosis technique.[15]

In conclusion, the incidence of *T. marneffei* infection in non-HIV-infected patients has been relatively low in recent years; however, it has shown a significant increase,[16] even in some healthy hosts.[17]Patients who are congenitally immunocompromised by a *STAT3* mutation may be among potential hosts. Finally, rapid diagnosis and early stage treatment are critical for improving the prognosis. The successful application of NGS can play an important role in rapid diagnosis, revealing the potential value of this technique in rapid etiological diagnosis. Further studies are required to explore the pathogenesis and mechanisms of infection in HIV-negative patients with *STAT3* mutation infected with *T. marneffei*.

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Limin Wang: Methodology; Project administration; Writing-review & editing.

Weizhong Jin: Formal analysis; Writing-original draft.

Chunming Jiang: Methodology; Supervision; Writing-review & editing.

Lihui Xu: Investigation; Writing-original draft.

Jianping Xu: Investigation; Resources; Writing-original draft.

Yue Li: Formal analysis; Software; Writing-original draft.

Liusheng Wang: Data curation; Formal analysis; Software; Writing-review & editing.

Hualiang Jin: Methodology; Software; Writing-review & editing.

Availability of data and materials

The sequencing data supporting our findings is contained within the manuscript and additional supporting files. The datasets used and/or analysed during the study are also available from the corresponding author on reasonable request.

Conflict of interest statement

The authors declare that there is no conflict of interest.

Consent to publish

Written informed consent was obtained from the patient for publication of this case report and all accompanying images.

Ethics approval and consent to participate

We identified this patient during routine clinical practice and obtained consent to take samples of venous blood and bronchoalveolar lavage fluid after elaborate information. Written informed consent was obtained from the patient for publication of this case report and all accompanying images. Involvement of the ethical committee of Hangzhou first people's hospital was considered unnecessary, since the project was not based on a study protocol.

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Supplemental material

The reviews of this paper are available via the supplemental material section.

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