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Case Report

Metagenomic next-generation sequencing identified a brain abscess caused by mixed oral anaerobe infection: A case report

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ABSTRACT

Fusobacterium vincentii brain abscesses are relatively rare. Here, we report our treatment of an anaerobic brain abscess caused by a mixed infection of *Parvimonas micra, Streptococcus constellatus, Fusobacterium vincentii*, and *Bacteroides heparinolyticus* diagnosed by metagenomic next-generation sequencing (mNGS). This is the first reported case of *Fusobacterium vincentii* in a brain abscess. This case highlights the possibility that oral anaerobic microbes can cause a brain abscess and demonstrates that mNGS has the potential to be deployed to provide rapid infection diagnosis and rationalize antimicrobial therapy for brain abscesses.

1. Introduction

A brain abscess is a rare, but potentially fatal, central nervous system infection [1]. Despite significant advancements in anaerobic culture technology, new antibiotics, and diagnostic imaging, the mortality rate remains high, reaching up to 10% [2].

Anaerobic bacteria can spread from nearby sources of infection, such as dental, sinus, and middle ear infections, to the central nervous system, ultimately leading to brain abscesses [3]. *Parvimonas micra* is a gram-positive anaerobic coccus that predominantly exists in the oral cavity and the gastrointestinal tract. *Parvimonas micra* has been identified as a causative agent of brain abscesses [4]. *Streptococcus constellatus* is generally considered a commensal species in the mouth and gut, while being frequently involved in pyogenic infections of the central nervous system and abdomen. *Fusobacterium nucleatum* is a gram-negative obligately anaerobic bacillus that is normally found in the oropharyngeal and upper respiratory tracts. *Fusobacterium nucleatum* is an important pathogen in abscesses [5].

In clinical settings, identifying the causes of brain abscesses can be aided by imaging techniques such as computed tomography and magnetic resonance imaging (MRI), as well as pathogen culture [6]. However, traditional culture methods face challenges in terms of appropriate sample collection, alongside anaerobic culture and isolation procedures [1,7]. Metagenomic next-generation sequencing (mNGS) is an alternative approach to routine culture that has the potential to be deployed for rapid infection diagnosis and determining rational antimicrobial therapies.

Here, we report a case of anaerobic infection diagnosed by metagenomic sequencing. We found a mixed infection of *Parvimonas micra, Streptococcus constellatus, Fusobac-*

Abbreviation: MRI, magnetic resonance imaging; mNGS, metagenomic next-generation sequencing.

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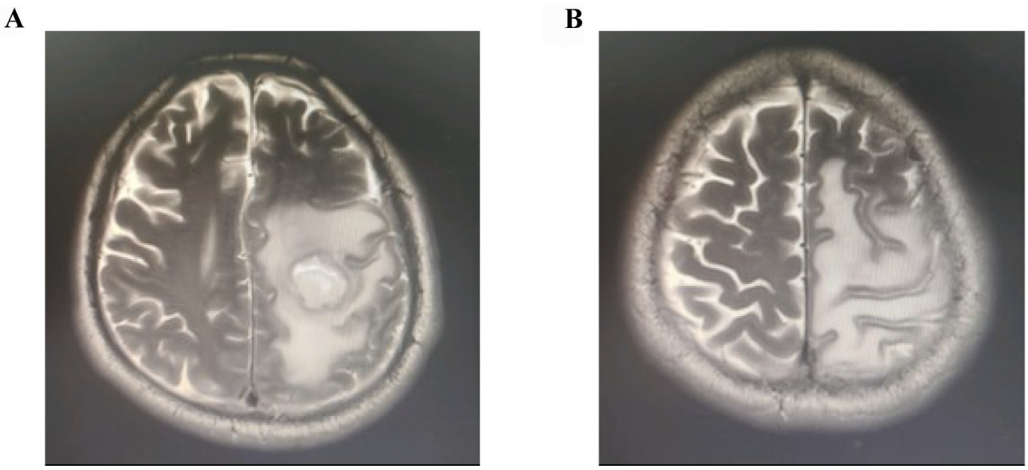

Fig. 1. (A) Axial T2-weighted fluid-attenuated image revealing a hyperintense lesion surrounded by a thin hypointense ring of about 2.9 cm × 2.7 cm × 3.3 cm in the frontal-parietal region. (B) Shallower cerebral sulci and fissures.

Table 1
Abnormal assay results of the patient.

Assay	Results	Reference value	Units
WBC	9.64	3.5–9.5	10^9/L
RBC	4.22	4.3–5.8	10^{12}/L
MCV	101.2	82–100	fL
PLT	389	125–350	10^9/L
RDW-SD	48.2	39–46	fL
C1q	156.4	159–233	mg/L
D-DIMER-IN	1.05	0–0.5	mg/L
APTT	38.2	25.1–36.5	s
APTT-radio	1.29	0.8–1.2	–
Fib	4.65	2.00–4.00	g/L
LYMPH	19.4	20–50	%
NEUT	75.2	40–75	%
EO	0.1	0.4–8.0	%
NEUT	7.25	1.8–6.3	10^9/L
EO	0.01	0.02–0.52	10^9/L
ALB	39.6	40–55	g/L
A/G	1.11	1.2–2.4	–
NA	135.6	137–147	mmol/L

Abbreviations: WBC, white blood cell; RBC, red blood cell; MCV, mean corpuscular volume; PLT, platelet; RDW-SD, red cell distribution width; APTT, activated partial thromboplastin time; LYMPH, lymphocyte; NEUT, neutrophils; EO, eosinophil; ALB, albumin; A/G, albumin/globulin; NA, natrium.

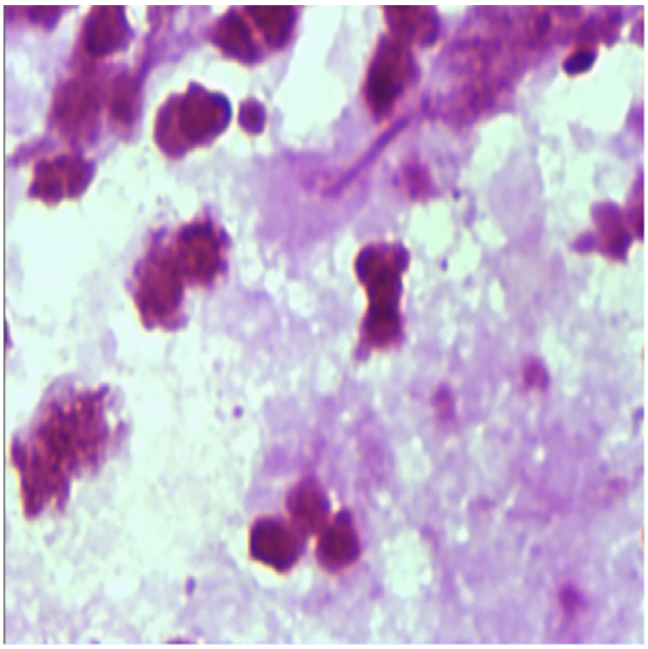

Fig. 2. Results of bacterial culture under an optical microscope.

terium vincentii, and *Bacteroides heparinolyticus* in the patient. mNGS reduced the detection time and detected drug resistance for timely treatment.

2. Case presentation

In July 2022, a 65-year-old man presented to Shandong Second Provincial General Hospital with the chief complaint of "hemiplegia on the right limbs for 10 days". The patient had unexplained discomfort in his right limbs 10 days before admission. MRI of the head revealed a lesion in the frontal-parietal region and multiple ischemic foci (Fig. 1). For further treatment, the patient was transferred to the Department of Neurosurgery of Shandong Provincial Hospital, Jinan, China on July 12, 2022.

Upon admission to Shandong Provincial Hospital, the patient's vital signs were recorded: temperature, 36.5°C; respiratory rate, 19 breaths/min; pulse, 76 beats/min; blood pressure, 157/107 mmHg. A chest X-ray ruled out any other abdominal or thoracic abscesses. The other abnormal assay results are outlined in Table 1.

As the patient's physical condition and clinical examination results were consistent with a need for surgery, we performed an exploratory and excision surgery to drain the abscess in the left frontal-parietal region. During surgery, a sample of pus was collected for laboratory analysis. Pus culture and mNGS were conducted to identify the pathogens and guide the appropriate clinical treatment.

The pathogens resulting from culture were *Parvimonas micra, Fusobacterium nucleatum, Streptococcus constellatus*, and anaerobic *Bacteroides* (Fig. 2). The drug resistance gene detected in the drug resistance analysis was

Table 2
Mapping features of high-abundance microbes and the best reference sequences.

Type	Species	Reference sequence	Identity (%)	Bases with coverage (%)	Average coverage depth	Maximum coverage depth
G⁺Obligate anaerobes	*Parvimonas micra*	LR134472.1	98.52	99.72	9.94	27
G⁻Obligate anaerobes	*Fusobacterium nucleatum*	CP071093.1	98.75	96.64	5.34	23
G⁻Obligate anaerobes	*Bacteroides heparinolyticus*	CP027234.1	99.81	53.76	0.92	10
G⁺Facultative anaerobes	*Streptococcus constellatus*	CP003859.1	99.84	42.46	0.82	13

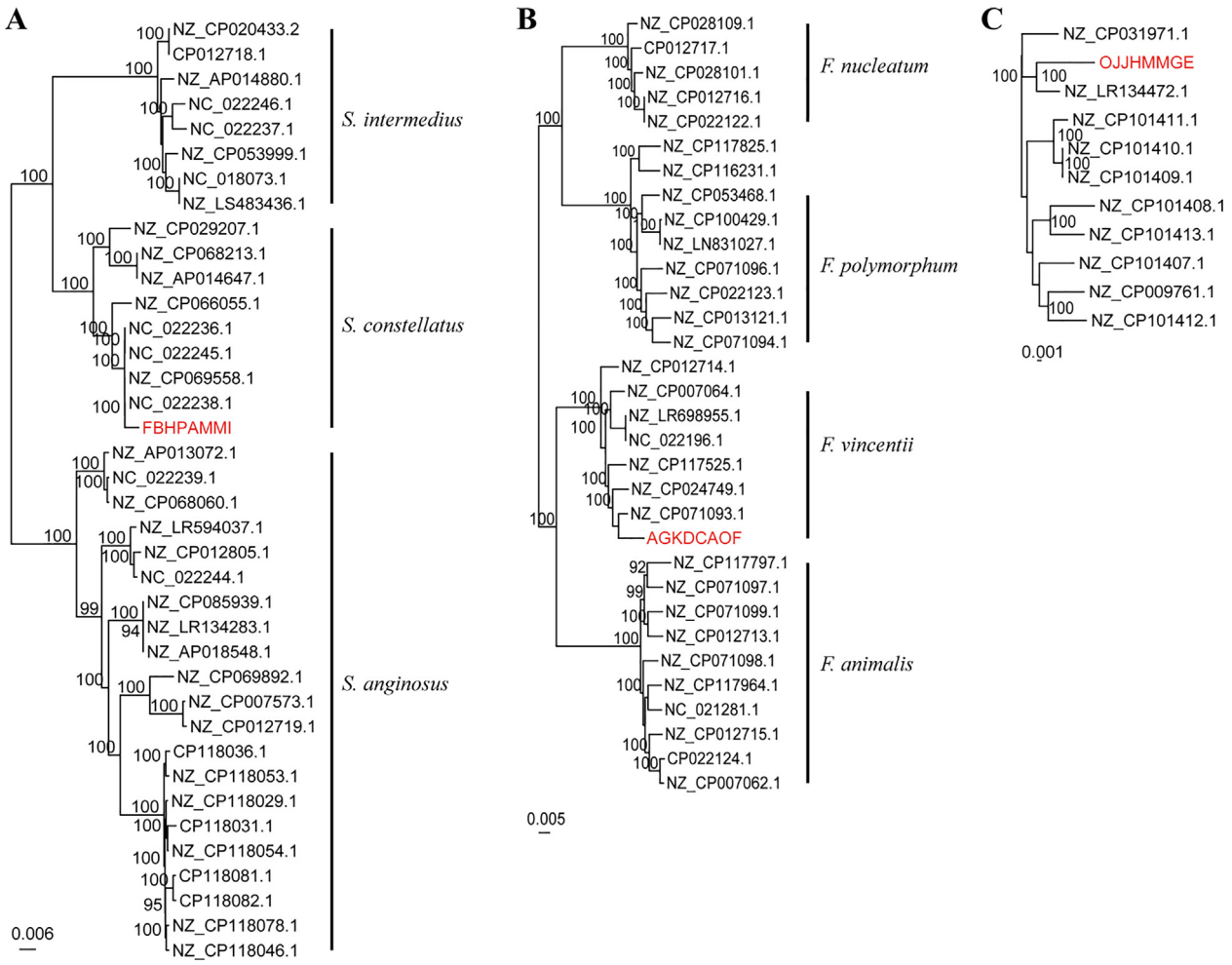

Fig. 3. Phylogenetic tree of single-copy lineal homologous genes recovered from a pus sample in a hospital in China. (A) Phylogenetic tree of 38 *Streptococcus anginosus* group strains (1 sequenced strain and 37 NCBI database strains). (B) Phylogenetic tree of 32 *Fusobacterium nucleatum* strains (1 sequenced strain and 31 NCBI database strains). (C) Phylogenetic tree of 11 *Parvimonas micra* strains (1 sequenced strain and 10 NCBI database strains).

Ermb_MLS. Streptogramin, macrolide, and lincosamide were ruled out as viable antimicrobial therapies. After exploratory and excision surgery, the patient received 4.5 g cefoperazone-sulbactam and 3 g piperacillin-tazobactam intravenously. After one day's treatment with antimicrobials, the symptoms of the infection further improved. The patient recovered his right limb strength after another 24 hours and was discharged from the hospital on the third day.

RNA reverse transcription, DNA extraction, RNA and DNA quantification, and nucleic acid standardization were processed with the MAPMI sample preparation kit (CapitalBio Co., Beijing, China) using the manufacturer's instructions. DNA libraries were constructed with enzymatic DNA fragmentation, end repair, adding adapters, and PCR amplification. The quality of the libraries was assessed using the Agilent 2100 system (Agilent Technologies, Santa Clara, California, USA). DNA sequencing was performed with an Ion PI chip on a BioelectronSeq 4000 platform (CapitalBio Co.). The bioinformatics analysis method is explained in the supplemental file.

The metagenomic sequencing generated 1,831,487 reads. After removing low-quality reads and human reads, we retained 658,816 clean reads for downstream analysis. We identified four species of anaerobic bacteria (*Parvimonas micra, Fusobacterium nucleatum, Bacteroides hepari-*

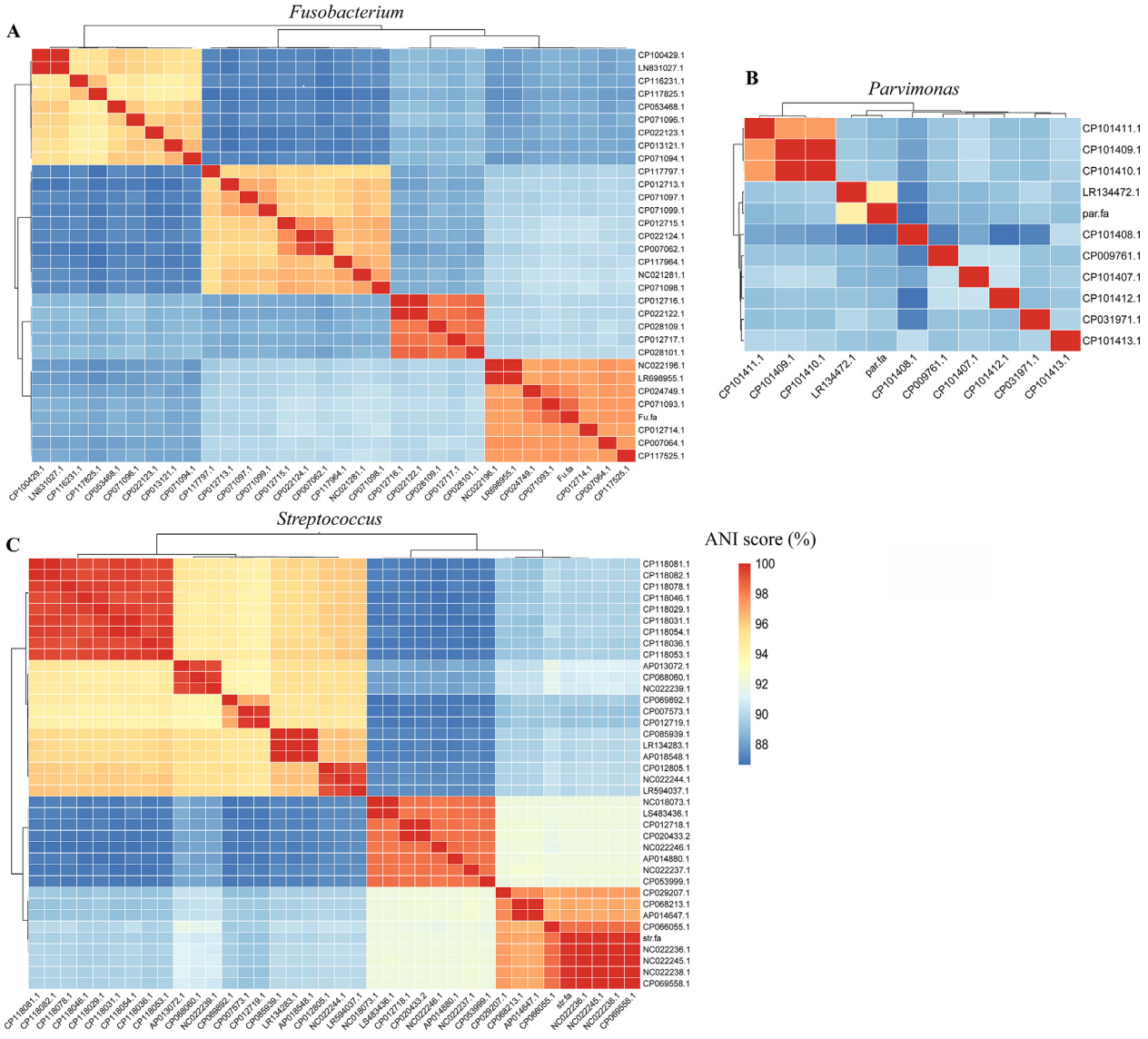

Fig. 4. Average nucleotide identity (ANI) analysis of sequenced and NCBI database strains. (A) ANI analysis of 1 sequenced *Fusobacterium vincentii* strain and 31 NCBI database strains. (B) ANI analysis of 11 *Parvimonas micra* strains. (C) ANI analysis of 38 *Streptococcus anginosus* group strains (1 sequenced strain and 37 NCBI database strains).

nolyticus, and *Streptococcus constellatus*) with abundances greater than 1% from the centrifuge results. All the consensus sequences of these anaerobic bacteria showed high similarity to their closest-related strains (Table 2).

Phylogenetic analysis for *Fusobacterium nucleatum* based on protein-coding sequences showed that all sequences were grouped into four distinct clusters. Our sequence fell within the branch of *Fusobacterium vincentii* strains (Fig. 3B). The ANI analysis (Fig. 4A) showed that our sequence had a 98.87% average nucleotide identity to *Fusobacterium vincentii* strain THCT14A3 (GenBank accession No. CP071093.1). Our sequence from *Parvimonas micra* phylogenetically fell within the branch of *Parvimonas micra* strains (Fig. 3C). The ANI analysis showed that our sequences had a 98.67% average nucleotide identity to *Parvimonas micra* strain NCTC11808 (GenBank accession No. LR134472.1) (Fig. 4B). Regarding *Streptococcus constellatus*, the phylogenetic tree showed that our sequence was in the cluster containing *Streptococcus constellatus* strains (Fig. 3A). Additionally, the ANI analysis showed that our sequence was similar to all *Streptococcus constellatus* strains (Fig. 4C).

3. Discussion

We encountered a case of a brain abscess caused by anaerobic bacteria and the treatment effect was satisfactory. In most cases, aerobic pathogens are considered responsible for brain abscesses. However, there has been increased detection of brain abscesses caused by anaerobic pathogens recently [8]. The actual incidence of brain abscesses caused by anaerobes may still be underestimated

because of the fastidious nature of these microorganisms, the specific growth conditions required to isolate anaerobic pathogens, and the challenges with identification [1]. Furthermore, the emergence of drug resistance among anaerobes highlights the urgent need for research on the diagnosis and treatment of anaerobic brain abscesses [9].

Akashi et al. reported a case of odontogenic brain abscesses. They identified the pathogens of the brain abscesses, which were *Streptococcus constellatus, Fusobacterium nucleatum*, and *Parvimonas micra*, from a bacteriological examination [10]. Odontogenic infections are a rare, but known, possible cause of brain abscesses via hematogenous spread. The patient had a 30-year history of smoking and poor oral hygiene, which may increase the risk of developing a brain abscess. This suggested that there was a high possibility that the patient's brain abscess was caused by an odontogenic infection of *Parvimonas micra, Streptococcus constellatus, Fusobacterium vincentii,* and *Bacteroides heparinolyticus. Fusobacterium nucleatum subsp. vincentii* was initially discovered by Vincent in 1896 and was reclassified as *Fusobacterium vincentii* in 2021 [11]. To the best of our knowledge, we are the first to report a brain abscess associated with *Fusobacterium vincentii*, suggesting that *Fusobacterium vincentii* could cause a brain abscess.

The current diagnosis of polymicrobial infections such as anaerobic brain abscesses still relies on traditional culture, which is time-consuming and has a poor positive rate. We applied mNGS to identify the pathogens. It took 6 days to obtain the results through culture, while mNGS took 1 day. At the genus level, the culture results were consistent with the results of the mNGS. However, mNGS provided more precise classifications of the bacterial species, including *Fusobacterium vincentii* and *Bacteroides heparinolyticus*. Our results demonstrated that mNGS has advantages of timeliness and accuracy in identifying pathogens of brain abscesses compared with traditional cultivation methods.

In conclusion, we reported a case of a *Fusobacterium vincentii* brain abscess diagnosed by mNGS. The patient had a co-infection of *Parvimonas micra, Streptococcus constellatus, Fusobacterium vincentii*, and *Bacteroides heparinolyticus*. With a series of treatments applied after the confirmed diagnosis, the patient's symptoms were relieved. These findings showed that mNGS may provide significant assistance when applied alongside conventional culture, highlighting the application potential of mNGS in diagnosing anaerobic infections.

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Author contributions

Y.F.X. and H.L. designed the study. H.L. and M.L. performed material preparation and data collection. Z.P.M., C.L., Y.H.W., H.L. and Y.F.X. analyzed and interpreted data. Z.P.M. drafted the manuscript. Y.F.X. reviewed and revised the manuscript. All authors were involved in the review of various previous versions of the manuscript. All authors have read and approved the final manuscript.

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Declaration of competing interest

The authors declare no conflicts of interest.

Data available statement

The patient information that supports the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Ethics statement

The Institutional Review Board for Medical Ethics and Research of the Jinan Centers for Disease Control and Prevention (CDC) reviewed and approved this project, and its conduct was consistent with applicable ID 2022-004.

Informed consent

Written informed consent was obtained from the patients for publication of this manuscript and any accompanying images.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.imj.2024.100109.

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