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Lack of significant ganglioside changes in *Slc17a5* heterozygous mice: Relevance to FSASD and Parkinson's disease

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ABSTRACT

Large population-based studies of Parkinson's disease (PD) have identified susceptibility genes, including *SLC17A5*. Biallelic mutations in *SLC17A5*, encoding the lysosomal sialic acid transporter sialin, cause the rare neurodegenerative disease, free sialic acid storage disorder (FSASD). To explore a potential biochemical link between FSASD and PD, we investigated ganglioside concentrations in a novel mouse model harboring the *Slc17a5* p.Arg39Cys (p.R39C) variant. Our analysis revealed no significant alterations in ganglioside concentrations in heterozygous p.R39C mice, warranting further studies into other potential links between PD and sialin defects.

1. Introduction

Parkinson's disease (PD), the second most common neurodegenerative disorder, is primarily characterized by the selective degeneration of dopaminergic neurons in the substantia nigra [1], leading to motor and non-motor neurological symptoms. While aging is the greatest risk factor for PD [2,3], genetic contributions to both familial (1–2 % of cases) and sporadic forms (98–99 % of cases) of the disease have been increasingly recognized [4]. Large-scale pathway analyses using polygenic risk scores have highlighted endosomal–lysosomal dysfunction as a key biological contributor to PD [5]. Moreover, Robak et al. (2017) identified a substantial burden of rare, likely pathogenic heterozygous variants in lysosomal storage disorder genes, including *SLC17A5*, *GBA*, *SMPD1*, *CTSD*, and *ASAH1*, in PD cases, suggesting that these genes may contribute to disease susceptibility [6]. Among these, *SLC17A5* encodes sialin (NCBI Gene: 26503), a lysosomal proton-coupled cotransporter responsible for exporting sialic acid and other acidic hexoses from lysosomes into the cytosol [7–11]. Pathogenic variants in *SLC17A5* cause free sialic acid storage disorder (FSASD; MIM#269920, MIM#604369) [12–15], a lysosomal storage disorder characterized by the accumulation of unconjugated "free" sialic acid in lysosomes, resulting in neurological dysfunction and multisystem involvement [10,16,17].

Seventeen non-synonymous *SLC17A5* variants predicted to be damaging based on CADD scores were identified in both PD cases and neurologically healthy individuals [6]. Two particular variants - p.Arg39Cys (p.R39C) and c.1111+1G > A - were exclusively found in individuals with PD [6] and have also been reported in cases of lysosomal free sialic acid storage disorder (FSASD) [12,13] (Supplementary Table 1). Notably, p.R39C is a founder missense variant, present in approximately 75% of documented FSASD cases [12,13]. Additionally, three variants—p.F432S, p.G383A, and p.G237E—were detected solely in PD cases [6], though these have not been reported in FSASD.

Sialic acids, including N-acetylneuraminic acid (Neu5Ac) in humans [18,19], are negatively charged monosaccharides involved in diverse biological processes, including immune regulation, cellular interactions, and neuronal function [20]. These molecules are most concentrated in the mammalian CNS, with 65% found in gangliosides (i.e., glycosphingolipids containing sialic acid residues), 32% in glycoproteins, and 3% as free sialic acid [21]. Most sialic acids are incorporated into gangliosides, while polysialic acid is attached to glycoproteins like NCAM in

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the CNS [22]. The primary pathway for catabolism of sialoglycoconjugates, including gangliosides, is lysosomal degradation, where sialidases (i.e., neuraminidases) remove terminal sialic acids from glycan chains [20]. Disruption of sialic acid homeostasis can impair neuronal function, synaptic transmission, and neural plasticity [23]—critical processes that are often compromised in neurodegenerative diseases, including PD [24,25].

Given that lysosomal dysfunction plays a key role in both PD and FSASD, and *SLC17A5* is both a proposed genetic risk factor for PD and a known cause of FSASD, we explored potential biochemical links between these disorders by examining changes in glycosphingolipids (GSLs). Impaired GSL metabolism has been increasingly implicated in neurodegeneration associated with PD [26,27], while studies in FSASD have shown decreased ganglioside turnover in patient fibroblasts, alongside elevated lysosomal enzyme levels despite normal enzyme activity, including sialidase in some studies [28–34]. Since SLC17A5 is critical for lysosomal sialic acid export, its dysfunction could impact ganglioside homeostasis, potentially contributing to PD pathogenesis. However, whether heterozygous *SLC17A5* variants (including p.R39C) affect ganglioside concentrations *in vivo* remains unknown. We therefore utilized a knock-in mouse model carrying the *Slc17a5* p.R39C variant to explore ganglioside concentrations in heterozygous *Slc17a5* p.R39C mice compared to wild-type mice. Additionally, given that PD is an age-related disorder and the p.R39C variant is proposed to be linked to PD risk, we investigated if aged heterozygous *Slc17a5* p.R39C mice exhibit more pronounced ganglioside changes than younger mice.

To investigate whether *SLC17A5* heterozygosity affects ganglioside homeostasis, we analyzed brain tissue from young and aged *Slc17a5* p.R39C heterozygous mice. Given that lysosomal ganglioside processing may be disrupted in FSASD and PD, we also assessed sialidase activity and measured systemic free sialic acid concentrations as an exploratory biomarker. By examining the biochemical impact of a PD-associated *SLC17A5* variant, this study provides key insights into potential lysosomal contributions to neurodegeneration.

2. Methods

2.1. Description of murine model

For this study, we utilized a knock-in mouse model carrying the human-equivalent *SLC17A5* p.R39C point mutation. Briefly, we humanized the corresponding sequence in the murine genome by CRISPR-Cas12a to alter two nucleotides (*Slc17a5* c.115C > T and c.117G > C) in exon 2 of the murine *Slc17a5* gene (NM_172773) to generate the p.R39C murine on a hybrid background (C57BL/6J-129S1/SvlmJ) (descriptive manuscript in preparation, [35]).

At three weeks of age, homozygous mice appeared smaller than wild-type littermates and showed tremors, ataxia, and seizures, with 25% succumbing to severe symptoms by 1.5 months, and nearly 50% dying by six months due to progressive ataxia and hindlimb paralysis. In contrast, heterozygous littermates showed no symptoms and remained visually indistinguishable from wild-type mice throughout their lifespan.

2.2. Genotyping of murine model

Genotyping was performed using TaqMan-based quantitative fluorescence PCR on genomic DNA extracted from tail biopsies of 21-day-old mice. Tail samples were collected and DNA was extracted using the Extract-N-Amp kit (Sigma-Aldrich) following the manufacturer's protocol. The samples were amplified using TaqMan Genotyping Master Mix (ThermoFisher Scientific) and analyzed on an ABI 7500 instrument (Applied Biosystems). The amplification was carried out with the following primers and probes: forward primer (CTCCATCTTAATTTCAGCTCCAGTGT), reverse primer (AAACCACAGAACGCCAAAATCG), VIC probe for wild-type allele detection (VIC – CTCTGCTCGGTACAACT – NFQ), and FAM probe for mutant allele detection (FAM – CTCTGCTTGCTACAACT – NFQ). Differences in the VIC and FAM fluorescence intensities distinguished the wild-type, *Slc17a5* p.R39C heterozygous, and homozygous genotypes.

2.3. Murine specimen collection

Mice were anesthetized with 1.25% tribromoethanol. Following anesthesia, a midline incision was made to expose the inferior vena cava (IVC) and urinary bladder. For the aged cohort, blood was collected from the IVC to obtain serum, transferred into 1.5 mL microcentrifuge tubes, and left to clot for 30 min. Samples were then centrifuged at 4°C for 20 min at 1500g, and the serum supernatant was transferred to fresh tubes and stored at −80°C. For the aged cohort, urine, if present, was aspirated directly from the urinary bladder using a syringe. Following blood and urine collection (if applicable), mice were perfused intracardially with 1X phosphate-buffered saline (PBS). The brain was rapidly dissected, removing the brainstem and bilateral olfactory bulbs. The left hemisphere was sectioned into the forebrain and cerebellum, flash-frozen in liquid nitrogen, and stored at −80 °C for downstream analyses.

2.4. Quantification of cellular GSLs

Glycosphingolipid (GSL) profiling in mouse forebrain and cerebellum tissues was conducted using published protocols [36]. Briefly, lipids were extracted overnight at 4°C using a chloroform-methanol mixture. GSLs were then purified using C18 columns (Telos). After elution, the GSL fractions were dried under a nitrogen stream at 42°C and digested with recombinant ceramide glycanase (rEGCase, prepared by Genscript and provided by Orphazyme) to release oligosaccharides from complex GSLs. The released glycans were fluorescently labeled with anthranilic acid (2AA), and excess 2AA was removed using DPA-6S SPE columns (Supelco). The purified 2AA-labeled oligosaccharides were separated and quantified by normal-phase high-performance liquid chromatography (NP-HPLC). A 2AA-labeled glucose homopolymer ladder (Ludger) was used to determine the glucose unit (GU) values for the HPLC peaks. Individual GSL species were identified based on their GU values (Supplementary Figs. 3A–B) and quantified by comparing the integrated peak areas to a known 2AA-labeled BioQuant chitotriose standard (Ludger). The results were subsequently normalized to protein content, which was determined using the bicinchoninic acid (BCA) assay.

We assessed a range of GSLs, including gangliosides, but due to their near-baseline levels, species such as lactosylceramide (Lac), GA2, and GA1 were unreliable for quantification and were excluded from further analysis and inclusion in "Total GSLs". As a result, gangliosides became the primary focus of this evaluation (Supplementary Figs. 3A–B).

2.5. Quantification of neuraminidase activities

Neuraminidase (NEU) activities were measured fluorometrically using a synthetic substrate, N-acetylneuraminic acid conjugated with the fluorophore 4-methylumbelliferone (4-MU), as previously described [37]. Two assays were performed, i.e., one to quantify the combined activity of NEU1, NEU3, and NEU4 (NEU1/3/4), and another to specifically measure cytosolic NEU2 activity. Both assays were incubated at 37°C for 3.16 h. Enzyme activities were determined by comparing fluorescence to a standard curve of unconjugated 4-MU and normalized to protein content as measured by BCA assay.

2.6. Quantification of free sialic acid

Neu5Ac, the most abundant mammalian sialic acid [38], was measured and is referred to as "sialic acid" in this report. Urinary and serum concentrations of free sialic acid from mice were quantified. 25 μL of urine or serum was analyzed using UPLC-MS/MS (Waters Acquity

I-Class; Xevo TQ-S MS/MS). Chromatographic separation was performed with a C18 reverse-phase column (Waters Acquity UPLC HSS T3), and detection was achieved through tandem mass spectrometry in selected reaction monitoring mode (electrospray ionization, negative ion mode). Quantification was carried out using stable isotope dilution and comparison to a standard curve. Urine free sialic acid concentrations were normalized to creatinine levels, measured by the alkaline picric acid method (Jaffé reaction). Serum free sialic acid concentrations were reported without further normalization.

2.7. Statistical analyses

Statistical analyses were conducted using an unpaired *t*-test between wild-type and p.R39C heterozygous mice using GraphPad Prism (GraphPad, version 10.0.3). Correlations were analyzed with Pearson correlation analysis. A two-sided *p*-value significance was set at 0.05.

3. Results

To investigate changes in ganglioside concentrations as a potential metabolic connection between PD and FSASD, we measured GSL levels and neuraminidase enzyme activities in both younger (mean age: 152 ± 9 days) and aged (mean age: 546 ± 70 days) wild-type and *Slc17a5* p.R39C heterozygous mice (see Supplementary Table 2 for cohort details). We selected these timepoints for several reasons. First, ganglioside levels are known to change with age [26]. Second, given that PD is an age-related disorder, the inclusion of aged mice was particularly relevant. Finally, ganglioside concentrations are known to be influenced by neurodegeneration [26], which generally manifests later in life.

In the aged cohort, we also quantified free sialic acid in urine and serum using UPLC-MS/MS; no significant differences were observed between genotypes (Supplementary Fig. 1).

3.1. GSL profiling of mouse forebrain and cerebellum

Quantitative analysis of GSLs isolated from the forebrain and cerebellum of younger and aged mice heterozygous for the *Slc17a5* p.R39C variant revealed no significant differences in GSL concentrations compared to wild-type controls (Fig. 1A–D). The primary brain gangliosides, GM1a, GD1a, GD1b, and GT1b [39], were present at comparable levels across both genotypes. Although no statistically significant changes were found, we noted a modest trend toward lower ganglioside concentrations in the cerebellum of aged heterozygous mice compared to wild-type controls (Fig. 1D). Given the established age-related decline in ganglioside metabolism in neurodegeneration [26], further studies in larger cohorts may be required to determine whether this trend represents a subtle biochemical effect of *SLC17A5* heterozygosity.

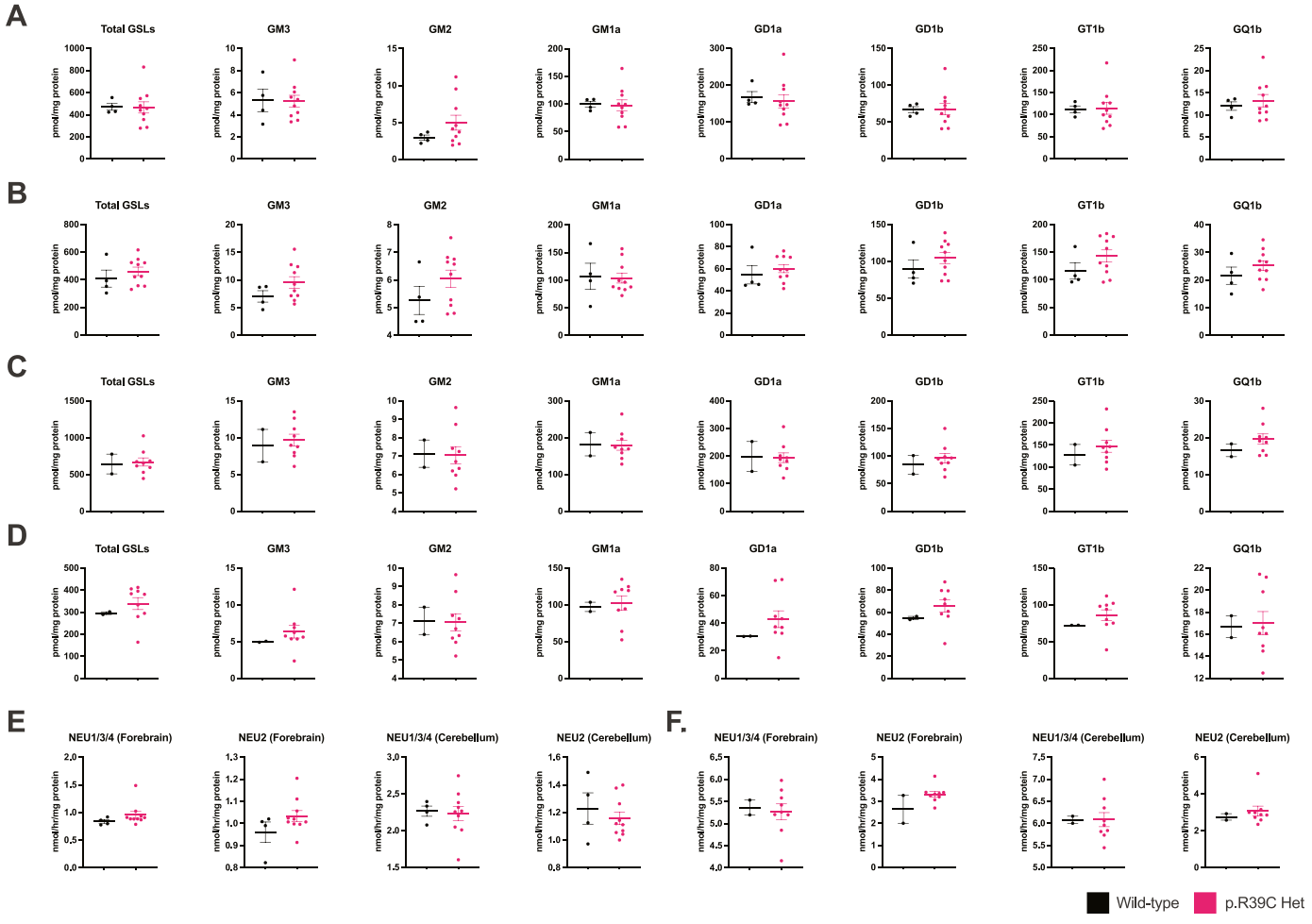

Fig. 1. Concentrations of total GSLs, individual GSL species, and neuraminidase enzyme activities in mouse forebrain and cerebellum. GSL concentrations in heterozygous *Slc17a5* p.R39C mice (pink) compared to wild-type mice (black) for: **(A)** younger mouse forebrain, **(B)** younger mouse cerebellum, **(C)** aged mouse forebrain, and **(D)** aged mouse cerebellum. Neuraminidase activity levels in **(E)** younger mouse forebrain and cerebellum and **(F)** aged mouse forebrain and cerebellum. For GSL measurements and neuraminidase enzyme screening, the younger mouse cohort consisted of 4 wild-type and 10 *Slc17a5* p.R39C heterozygous mice, while the aged cohort included 2 wild-type and 9 *Slc17a5* p.R39C heterozygous mice. Mean ± SEM; unpaired *t*-test.

3.2. Correlation of free sialic concentrations and ganglioside concentrations

We next explored whether ganglioside concentrations correlated with urinary and serum free sialic acid concentrations, as sialic acid homeostasis and ganglioside metabolism are functionally linked. No significant correlations were observed in the forebrain, but we found a borderline significant positive correlation between urinary free sialic concentrations and ganglioside concentrations in the mouse cerebellum (Supplementary Figs. 2A–D). This unexpected trend suggests that systemic sialic acid availability may influence cerebellar ganglioside metabolism, although further studies are needed to confirm this finding.

3.3. Neuraminidase activity in mouse brain

To determine whether sialin dysfunction impacts lysosomal sialidase activity, we measured NEU1/3/4 and cytosolic NEU2 activities in brain homogenates from heterozygous p.R39C and wild-type mice at both younger and aged timepoints. NEU1/3/4 and NEU2 activities remained unchanged in p.R39C heterozygous mice relative to wild-type controls (Fig. 1E–F). However, neuraminidase activity levels were significantly higher in the aged cohort compared to the younger cohort in both genotypes (Fig. 1E–F).

These results indicate that heterozygous *Slc17a5* p.R39C mice do not exhibit major defects in lysosomal ganglioside processing, at least under basal conditions. However, the age-dependent increase in sialidase activity warrants further investigation, particularly in the context of age-related neurodegeneration and PD pathogenesis.

4. Discussion

Given the proposed role of *SLC17A5* as both a genetic risk factor for Parkinson's disease and a monogenic cause of FSASD, our study aimed to explore potential links between these neurodegenerative disorders by analyzing ganglioside concentrations and sialic acid homeostasis. While ganglioside concentrations has been increasingly implicated in neurodegeneration [26,26,27,40], our results do not show a significant effect of *Slc17a5* heterozygosity on brain ganglioside levels (including GM1a, GD1a, GD1b, and GT1b) in mice. The absence of significant changes in ganglioside concentrations, even in aged heterozygous mice, suggests that the p.R39C variant alone may not trigger GSL accumulation or substantial alterations in lysosomal glycosphingolipid processing. However, this does not exclude a pathogenic role for *SLC17A5* variants in PD, as additional genetic or environmental stressors may be required to reveal impacts on lysosomal function, as seen in *gba* point mutation mouse models of PD [41].

Interestingly, urinary free sialic acid concentrations correlated positively with cerebellar ganglioside concentrations in aged mice, likely suggesting a possible age-related regulation of sialic acid metabolism and ganglioside turnover. While this finding may be incidental, it warrants further investigation, particularly given the high concentration of sialic acid in the brain and their role in neuronal stability [42,43]. Future studies should also measure Neu5Gc, the predominant sialic acid in peripheral tissues of young and aged mice and correlate these levels with brain gangliosides where Neu5Ac predominates [44].

Since ganglioside catabolism occurs via lysosomal neuraminidases [20], we assessed neuraminidase activities (NEU1/3/4 and NEU2) in the p.R39C heterozygous model. Consistent with the GSL findings, no significant differences were observed between genotypes at either time point, reinforcing the idea that *Slc17a5* heterozygosity alone does not impair lysosomal ganglioside degradation. Interestingly, neuraminidase activity was higher in aged mice, consistent with prior reports of age-related enzymatic shifts [45].

Several studies have underscored the role of lysosomal dysfunction in PD susceptibility and pathogenesis, with particular emphasis on β-glucocerebrosidase, the enzyme encoded by the *GBA1* gene [46,47]. Recent evidence suggests that ganglioside metabolism may play a distinct role in the pathophysiology of PD, particularly in the context of GBA1-related lysosomal dysfunction. Measurement of brain gangliosides across idiopathic PD, PD-GBA, and neurologically healthy controls reveals slightly elevated ganglioside concentrations in the idiopathic PD group, and a more pronounced and statistically significant increase in the PD-GBA group [48], implicating a role of ganglioside in disease progression [50]. This is likely because GBA1 is central to the lysosomal catabolism of the GSL, glucosylceramide whereas SLC17A5 is not directly involved in glycosphingolipid metabolism, which may explain the lack of significant changes in ganglioside concentrations in our mouse model. To gain a clearer understanding of these differences, further studies are needed to compare human brain samples from idiopathic PD and controls with those from PD-SLC17A5, a group that includes PD patients carrying *SLC17A5* variants.

This study has limitations, including limited availability of wild-type mice for the aged cohort due to constraints in breeding heterozygous mice, which reduced statistical power. While we observed no statistical significance, we cannot rule out the possibility that a larger sample size could reveal biochemical alterations. Next, while the younger cohort included both male and female mice, the aged cohort only included male mice to mirror the disproportionate incidence of PD in aging males [2].

Our study focused on global ganglioside levels in mouse brain tissues as an initial approach to assess broad changes, but did not distinguish between plasma membrane-bound and lysosomal ganglioside pools. Subcellular fractionation could provide additional critical insights, and emerging technologies like tagless Lyso-IP [49] hold promise for addressing this limitation. Further, our study focused on ganglioside concentrations at only two specific timepoints and two specific brain regions in the p.R39C heterozygous mice. Expanding this approach to additional timepoints and PD-relevant brain regions, such as the substantia nigra or basal ganglia, could uncover more localized or progressive changes in ganglioside homeostasis. Additionally, while we focused on ganglioside concentrations, future studies could incorporate flux analysis and a broader screening of lysosomal hydrolases involved in GSL catabolism. Future studies may also benefit from using a heterozygous *Slc17a5* knock-out model [50] to remain *SLC17A5* variant-agnostic. Beyond gangliosides, oligosaccharide profiling could offer complementary insights into sialic acid metabolism in FSASD and PD.

Despite the limitations, this study represents the first attempt to investigate a biochemical connection between PD and FSASD in the context of *SLC17A5* heterozygosity, given its association with both disorders. Although lysosomal dysfunction is a prominent feature of both conditions, our findings do not support a shared pathomechanism involving changes in ganglioside concentrations in heterozygous p.R39C carriers. Collectively, this study underscores the complexity of age-related metabolic changes in the brain and highlights the necessity for further studies to clarify the genetic overlap between these disorders in larger PD case-control studies.

CRediT authorship contribution statement

Marya S. Sabir: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Mahin S. Hossain:** Writing – review & editing, Methodology, Investigation. **Laura Pollard:** Writing – review & editing, Investigation, Data curation. **Marjan Huizing:** Writing – review & editing, Data curation. **William A. Gahl:** Writing – review & editing, Supervision, Funding acquisition. **Frances M. Platt:** Writing – review & editing, Supervision, Resources, Funding acquisition, Formal analysis, Conceptualization. **May Christine V. Malicdan:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Formal analysis, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrep.2025.101979.

Data availability

Data will be made available on request.

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