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REPORT

Recurrent PTPRZ1-MET fusion and a high occurrence rate of MET exon 14 skipping in brain metastases

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Abstract

Identifying molecular features is an essential component of the management and targeted therapy of brain metastases (BMs). The molecular features are different between primary lung cancers and BMs of lung cancer. Here we report the DNA and RNA mutational profiles of 43 pathological samples of BMs. In addition to previously reported mutational events associated with targeted therapy, *PTPRZ1-MET*, which was previously exclusively identified in glioma, was present in two cases of BMs of lung cancer. Furthermore, MET exon 14 skipping may be more common (6/37 cases) in BMs of lung cancer than the frequency previously reported in lung cancer. These findings highlight the clinical significance of targeted DNA plus RNA sequencing for BMs and suggest *PTPRZ1-MET* and MET exon 14 skipping as critical molecular events that may serve as targets of targeted therapy in BMs.

KEYWORDS
brain metastases, lung cancer, MET exon 14 skipping, molecular testing, PTPRZ1-MET fusion

1 | INTRODUCTION

Brain metastases (BMs) are the most common malignant intracranial tumors in adults, and the most common primary tumors of BMs are lung cancers.[1] With the development of targeted therapies, the overall survival of cancer patients is improving, and the incidence of BMs is also increasing.[2] Identifying molecular features of BMs has become essential for the effective management and treatment of BMs.[3,4] Because of the limited availability of BM samples, determining the molecular features for BMs is largely based on molecular testing of primary tumors or fluid biopsy. However, the molecular features are different between primary lung cancers and BMs.[5,6] Therefore, evaluating molecular events in BM samples is critical for effective treatment.

In this study, we examined the molecular characteristics of pathological samples of BMs by determining the DNA and RNA mutational profiles of 43 cases of BM using the Oncomine™ Focus Assay.[7]

Rui-Chao Chai and Xing Liu contributed equally to this work.

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2 | MATERIALS AND METHODS

2.1 | Cases and clinical samples

This study included 43 cases with BMs and 1 case with glioblastoma that underwent surgical resection at Beijing Tiantan Hospital. Freshly prepared (within 2 weeks after surgery) formalin-fixed, paraffin-embedded (FFPE) samples were analyzed using the Oncomine™ Focus Assay (Thermo Fisher), as previously reported.[7] The average amplicon coverage was above 5000x. The study protocol was approved by the Institutional Review Board and Ethics Committee of Beijing Tiantan Hospital.

2.2 | PCR and Sanger sequencing

PTPRZ1-MET (ZM) was detected by PCR and Sanger sequencing. The primer sequences are as follows: forward 5′-TGCCG CCTGGATAAACCTCTC-3′ and reverse 5′-CGGTGAAGTTGGGAA GCTGA-3′. The PCR reaction was run for 50 cycles of 94°C for 30 s, 58°C for 30 s, and 72°C for 30 s, followed by final extension at 72°C for 5 min. PCR products (166 bp) were extracted from an agarose gel (1.5%) after electrophoresis and verified by Sanger sequencing.

2.3 | Immunohistochemistry

Immunohistochemistry (IHC) was performed as previously described.[8,9] The primary antibodies included anti-c-MET antibody (1:500; Abcam); anti-p-MET antibody (1:200; CST); anti-Ki-67 antibody (1:100; ZSGB-Bio), anti-TTF-1 antibody, (MAB-0599; MAB Technology), anti-CK antibody (kit-0009; MAB Technology), anti-GFAP antibody (ZSGB-Bio), and anti-Syn antibody (ZA-0506; ZSGB-Bio).

3 | RESULTS AND DISCUSSION

We determined the DNA and RNA mutational profiles of one glioblastoma and 43 BM cases, including three BMs of breast cancer, one BM of colon cancer, one BM of rectal carcinoma, one BM of maxillary sinus, and 37 BMs of lung cancer. The case characteristics are summarized in Table 1.

We detected single-nucleotide mutations, gene copy number variations, and gene fusions in glioblastoma and BMs (Figure 1 and Table 2). Common mutational events, including *EGFR* amplification, $EGFR^{vIII}$, and *FGFR-TACC3* fusion, were detected in the glioblastoma case. *ERBB2* amplification, *MYC* amplification, and $EGFR^{vIII}$ were detected in the BMs of breast cancers. *MYC* amplification was observed in the BM of maxillary sinus. *KRAS* mutation and *FGFR1* amplification were detected in the BM of colon cancer. We also detected common mutations in the BMs of lung cancer, including *EGFR* P.L858R, *EGFR* exon 19 fragment deletion, *EGFR* p.T790M, *KRAS* p.G12D, *EGFR* amplification, *EML4_ALK* (E13A20), and others (Figure 1A and Table 2).

Remarkably, we detected ZM in two of 37 BMs of lung cancer (Figure 1A and Table 2). The breakpoints of ZM in both cases are exon 1 of *PTPRZ1* to exon 2 of *MET* (hg19, chromosome 7:121513611 to chromosome 7:116339124) (Figure 1B). We validated the presence of ZM in FFPE samples of the two cases by gel-based PCR and Sanger sequencing (Figure 1C).

The first case (Lung_13), a 56-year-old woman, received pneumonectomy of the left upper lung, gamma knife therapy, and craniotomy of the right frontal lobe 4 years previously. The previous BM harbored *EGFR* exon 19 deletion and was treated with Tarceva. A lesion was detected in the left frontal lobe 2 months before surgery, and the patient was treated with Ametinib (Figure 2A). The surgical specimen was diagnosed as BM from lung adenocarcinoma with acinar and papillary growth pattern and was positive for pan-CK and TTF-1 in IHC.

Molecular testing of the surgical specimen showed the coexistence of *EGFR* p.E746_A750 (exon 19) deletion (mutant allele frequency [MAF]: 73.29%), p.T790M (MAF: 39.81%), *EGFR* amplification, $EGFR^{vIII}$, ZM, and *FGFR1-TACC1* (Table 2). The MAF of *EGFR* mutations suggests *EGFR* pT790M was a later event than *EGFR* pE746_A750 (exon 19) deletion. Previous studies showed that MET amplification and METex14 could be secondarily activated as a resistance mechanism for EGFR-targeted therapy.[10-12] Here, ZM might be secondarily activated after EGFR-targeted therapy.

The second case (Lung_15), a 50-year-old man, was admitted to the hospital with headache and dizziness for 1 month. Preoperative MRI showed a space-occupying lesion in the left cerebellar hemisphere of the brain (Figure 2B). CT revealed a mass in the lower lobe of the left lung. Postoperative pathological examination of the brain revealed a metastatic tumor from lung, with active proliferation, positive immunostaining for pan-CK, and diffuse nuclear TTF-1 expression. These findings suggest that ZM can also occur in untreated BMs of lung cancer.

ZM is an oncogenic variant of the *MET* gene that we previously identified in adult gliomas.[13] The breakpoints identified in this study are consistent with our findings in glioma. ZM was proven to be a therapeutic target for secondary glioblastoma and infantile glioma.[14,15] To the best of our knowledge, ZM has never been reported in other tumor types. ZM was only detected in glioma in the cancer genome atlas (TCGA) pan-cancer gene fusion dataset (Figure S1).

METex14 was detected in 16.2% (95% confidence interval [CI]: 6.2% to 32.0%) of the 37 BMs of lung cancer. This is higher than the rates reported in lung adenocarcinoma (approximately 3%) and other lung neoplasms (approximately 1%-2%).[16] METex14 was proven to be a valuable therapeutic target in clinical trials of non–small cell lung cancer (including BMs).[7,12] We found that METex14 occurs at a higher proportion (14%) in secondary glioblastomas than in pan-glioma (0.4%).[14,16] METex14 was also detected at a higher proportion in BMs of lung cancer in our study compared with primary lung cancer in previous reports. One possible reason is the molecular divergence between BMs and primary tumors,[5] and METex14 tends

TABLE 1 Clinical characteristics of cases in this study

Sample ID	Sex	Age	Lobe	Lateral	Primary tumor
Breast_1	Female	29	Cerebellum	Right	Breast cancer
Breast_2	Female	44	Parietal	Right	Breast cancer
Breast_3	Female	47	Cerebellum	Right	Breast cancer
Colon_1	Female	48	Cerebellum	Right	Colon cancer
GBM	Male	45	Frontal/temporal	Right	Glioma
Lung_1	Female	49	Frontal	Right	Lung cancer
Lung_2	Male	62	Occipital	Left	Lung cancer
Lung_3	Male	72	Frontal	Left	Lung cancer
Lung_4	Male	57	Frontal (multiple lobes)	Right	Lung cancer
Lung_5	Male	55	Frontal	Left	Lung cancer
Lung_6	Male	56	Frontal	Right	Lung cancer
Lung_7	Male	51	Occipital	Right	Lung cancer
Lung_8	Male	52	Frontal/parietal	Left	Lung cancer
Lung_9	Female	65	Frontal	Left	Lung cancer
Lung_10	Male	76	Occipital	Left	Lung cancer
Lung_11	Male	58	Temporal/occipital	Right	Lung cancer
Lung_12	Female	53	Frontal	Left	Lung cancer
Lung_13	Female	56	Frontal/parietal	Left	Lung cancer
Lung_14	Female	49	Frontal/temporal	Left	Lung cancer
Lung_15	Male	50	Cerebellum	Left	Lung cancer
Lung_16	Male	56	Cerebellum	Right	Lung cancer
Lung_17	Male	59	Parietal/occipital	Right	Lung cancer
Lung_18	Male	58	Cerebellum	Left	Lung cancer
Lung_19	Male	57	Temporal/parietal	Right	Lung cancer
Lung_20	Male	57	Occipital	Right	Lung cancer
Maxillary_1	Male	47	Frontal	Right	Maxillary sinus
Rectal_1	Female	55	Frontal	Bilateral	Rectal carcinoma

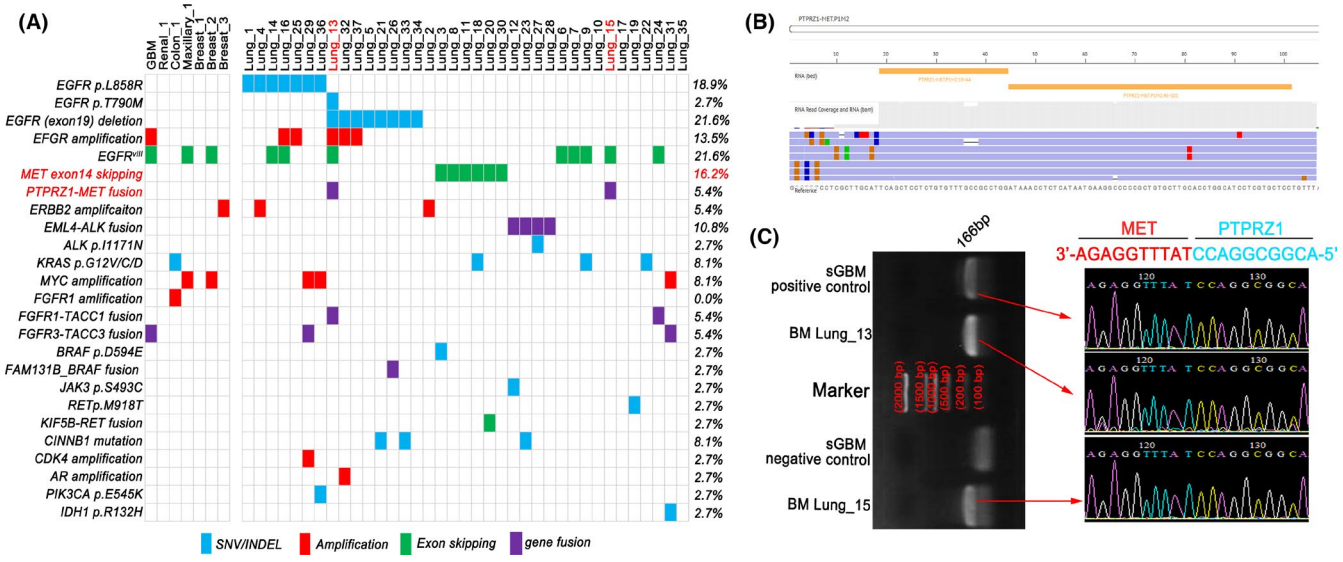

FIGURE 1 Molecular characteristics of brain metastases. A, The DNA and RNA mutational profile in formalin-fixed, paraffin-embedded (FFPE) samples of 43 brain metastases and a glioblastoma. B, Schematic fusion configuration of case Lung_15 showing PTPRZ1-MET fusion. C, PCR products of cases by PTPRZ1-MET (exon1-exon2) specific primers and Sanger sequencing results

TABLE 2 Molecular testing results of cases in this study

Case ID	Molecular characteristic (copy number/fusion format/mutant allele frequency)
GBM	EGFR amplification (29.54); EGFR_EGFR (E1E8); FGFR3-TACC3 (F17T7); FGFR3-TACC3 (F17T8); FGFR3-TACC3 (F17T9)
Rectal_1	No alterations
Colon_1	KRAS p.G12V (36.19%); FGFR1 amplification (5.74)
Maxillary_1	MYC amplification (5.84); EGFR_EGFR (E1:E8)
Breast_1	No alterations
Breast_2	MYC amplification (8.94); EGFR_EGFR (E1:E8)
Breast_3	ERBB2 amplification (32.33)
Lung_1	EGFR P.L858R (50.33%)
Lung_2	ERBB2 amplification (9.57)
Lung_3	BRAF p.D594E (61.59%); MET_MET (M13M15)
Lung_4	EGFR p.L858R (70.67%); ERBB2 amplification (6.31)
Lung_5	EGFR p.E746_A750 (exon 19) del (38.47%)
Lung_6	EGFR_EGFR (E1E8)
Lung_7	EGFR_EGFR (E1E8)
Lung_8	MET_MET (M13:M15)
Lung_9	KRAS p.G12D (13.94%); EGFR_EGFR (E1E8)
Lung_10	No alterations
Lung_11	MET_MET (M13:M15)
Lung_12	EML4_ALK (E13A20); JAK3 p.S493C (5.01%)
Lung_13	EGFR p.T790M (39.81%); EGFR p.E746_A750 (exon 19) del (73.72%); EGFR amplification (8.03); EGFR_EGFR (E1:E8); PTPRZ1_MET (P1M2); FGFR1_TACC1 (F17T7)
Lung_14	EGFR p.L858R (15.64%); EGFR_EGFR (E1:E8)
Lung_15	EGFR_EGFR (E1E8); PTPRZ1_MET (P1M2)
Lung_16	EGFR p.L858R (94.80%); EGFR amplification (12.39); EGFR_EGFR (E1:E8)
Lung_17	No alterations
Lung_18	KRAS p.G12C (53.1%); MET_MET (M13M15)
Lung_19	RET p.M918T (52.36%)
Lung_20	MET-MET (M13M15); KIF5B-RET (K15R12)
Lung_21	EGFR p.E746_A750 (exon 19) del (67.25%); CTNNB1 p.S33F (12.53%)
Lung_22	KRAS p.G12V (44.54%)
Lung_23	EML4_ALK (E6A20); CTNNB1 p.S45F (35.39%)
Lung_24	FGFR1_TACC1(F17T7); EGFR_EGFR (E1:E8)
Lung_25	EGFR P.L858R (61.71%); EGFR amplification (6.56)
Lung_26	EGFRp.E746_P753 (exon 19) delinsVS (51.29%); FAM131B_BRAF (F2B9)
Lung_27	EML4_ALK (E6A20); ALK p.I1171N (20.1%)
Lung_28	EML4_ALK (E6A20)
Lung_29	EGFR P.L858R (44.31%); FGFR3_TACC3 (F17T11); MYC amplification (5.93); CDK4 amplification (13.34)
Lung_30	MET_MET (M13M15)
Lung_31	IDH1 p.R132H (41.55%); FGFR3_TACC3 (F17T11); MYC amplification (7.86)
Lung_32	EGFR p.E746_A750 (exon 19) del (94.66%); AR amplification (9.43); EGFR amplification (15.29)
Lung_33	EGFR p.L747_P753 (exon 19) delinsS (52.16%); CTNNB1 p.S37C (40.1%)
Lung_34	EGFR p.L747_T751 (exon 19) del (51.78%)
Lung_35	No alterations
Lung_36	EGFR P.L858R (12.56%); PIK3CA p.E545K (16.3); MYC amplification (7.91)
Lung_37	EGFR p.L747_P753 (exon 19) delinsS (15.87%); EGFR amplification (15.20)

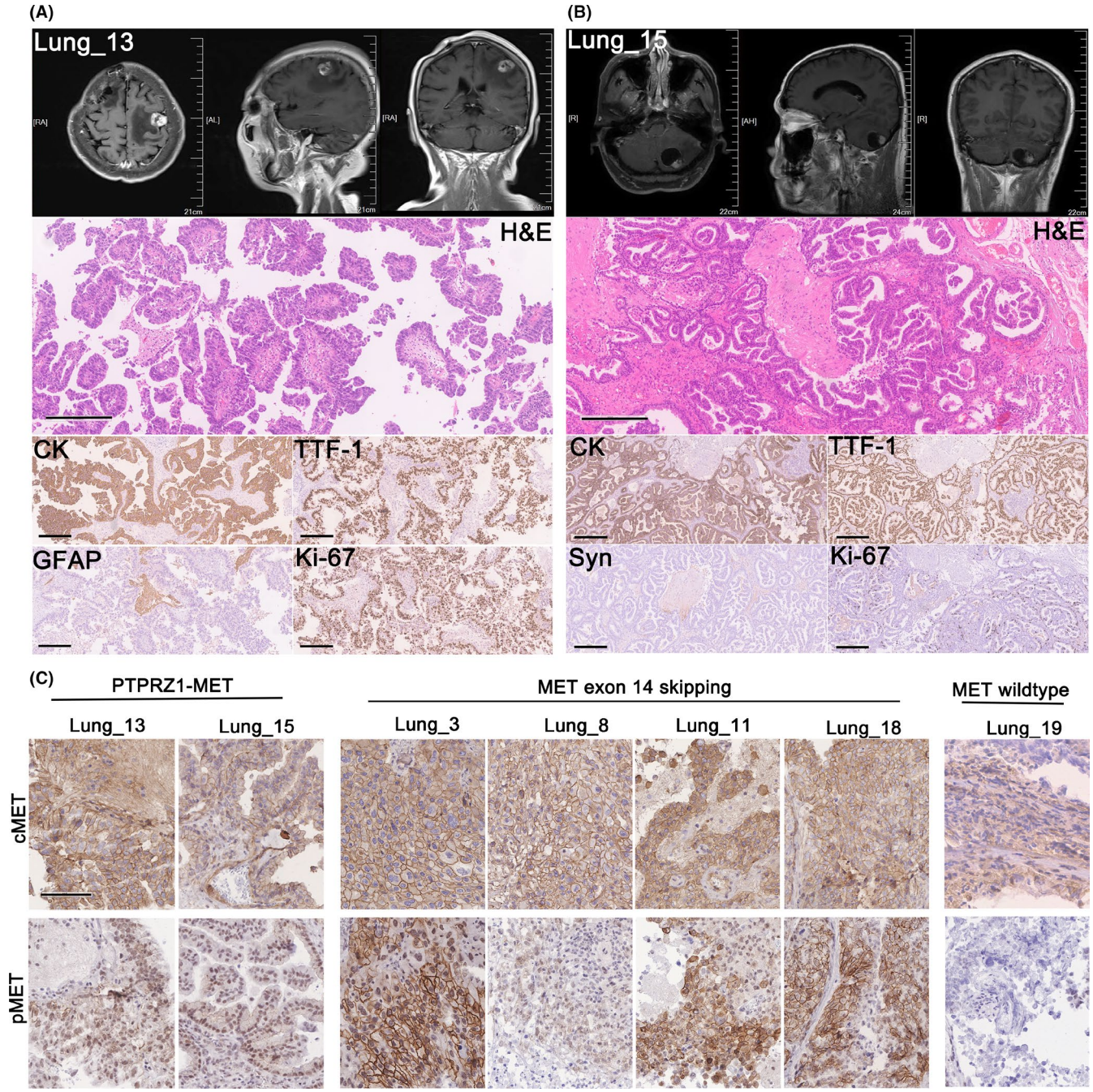

FIGURE 2 Images and pathological features of cases with PTPRZ1-MET fusion. A, B, T1 contrast-enhanced magnetic resonance imaging (MRI) and immunohistochemistry (IHC) features of brain metastases (BMs) Lung_13 (A) and Lung_15 (B). Bar = 300 μm. C, IHC results of c-MET and p-MET of BMs harboring PTPRZ1-MET, MET exon 14 skipping, or wildtype-MET. Bar = 100 μm

to occur in BMs of advanced lung cancer. Another possible reason is that the occurrence rate of METex14 is underestimated in the DNA-based molecular testing, and RNA-based testing is challenged in fluid biopsy.[12] The incidence of METex14 in lung cancer BMs needs validation in a larger cohort.

ZM enhances MET signaling activation through autophosphorylation of MET.[17] METex14 also causes sustained activation of MET by blocking the ubiquitin-mediated degradation of MET.[12] We performed IHC in the two cases harboring ZM and the four cases (with sufficient materials) harboring METex14; a case with wild-type MET was used as a comparison. Strong positive c-MET and positive p-MET signals were observed in cases harboring ZM or METex14, but not in the case with wild-type MET (Figure 2C). These results suggest that ZM and METex14 are critical molecular events that may represent therapeutic targets for BMs.

Notably, we observed that METex14 and *EGFR* mutations were mutually exclusive in the 37 BMs of lung cancer. These findings suggested MET RNA variants were another important RTK signaling–activated driver event, other than EGFR mutations, in BMs of lung cancer. Other fusions, including *EML4_ALK*,

FGFR1-TACC1, *FGFR3-TACC3*, *FAM131B_BRAF*, and *KIF5B-RET*, were also detected in BM samples. Thus, more attention should be paid to the role of RNA splicing variations or gene fusions in BMs, and methods that can evaluate RNA variations in BMs in clinical practice are warranted.

In summary, we demonstrated that ZM, which was previously identified in glioma, also occurs in BMs of lung cancer. Additionally, the proportion of METex14 in BMs of lung cancer may be higher than that previously reported in primary lung cancer. Molecular testing (including RNA testing) of BM samples could provide more precise information for targeted therapy of cancers with BMs.

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DISCLOSURE

All authors report no conflict of interest.

DATA AVAILABILITY STATEMENT

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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