Research article

Tau mRNA 3'UTR-to-CDS ratio is increased in Alzheimer disease

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HIGHLIGHTS

- Healthy mouse and human brain show a balanced 3'UTR-to-CDS *MAPT* ratio.
- *MAPT* mRNA is decreased in Alzheimer disease, whereas tau protein is increased.
- Neuronal markers are low in Alzheimer brain, in contrast to glial cell markers.
- Alzheimer patient brains have an increased 3'UTR-to-CDS *MAPT* ratio.

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ABSTRACT

Neurons frequently show an imbalance in expression of the 3' untranslated region (3'UTR) relative to the coding DNA sequence (CDS) region of mature messenger RNAs (mRNA). The ratio varies among different cells or parts of the brain. The Map2 protein levels per cell depend on the 3'UTR-to-CDS ratio rather than the total mRNA amount, which suggests powerful regulation of protein expression by 3'UTR sequences. Here we found that *MAPT* (the microtubule-associated protein tau gene) 3'UTR levels are particularly high with respect to other genes; indeed, the 3'UTR-to-CDS ratio of *MAPT* is balanced in healthy brain in mouse and human. The tau protein accumulates in Alzheimer diseased brain. We nonetheless observed that the levels of RNA encoding *MAPT*/tau were diminished in these patients' brains. To explain this apparently contradictory result, we studied *MAPT* mRNA stoichiometry in coding and non-coding regions, and found that the 3'UTR-to-CDS ratio was higher in the hippocampus of Alzheimer disease patients, with higher tau protein but lower total mRNA levels. Our data indicate that changes in the 3'UTR-to-CDS ratio have a regulatory role in the disease. Future research should thus consider not only mRNA levels, but also the ratios between coding and non-coding regions.

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1. Introduction

Tau, a microtubule-associated protein encoded by the *MAPT* gene, is involved in multiple neuropathologies. Tau is expressed

in brain, specifically in neuronal cells. In the adult human brain, the *MAPT* gene is composed of 16 exons on chromosome 17q21 that give rise to six tau isoforms, which are generated by alternative splicing of exons 2, 3 and 10 [1,2]. The *MAPT* gene is transcribed into its nuclear RNA which, after intron splicing and polyadenylation, becomes *MAPT* messenger RNA (mRNA) that bears its 5'UTR (untranscribed region), the coding exons (CDS) and its 3'UTR region; this transcription unit is a single entity that will be translated into tau protein.

In its normal physiological function, tau is differentially expressed during neuron development [3]. In young neurons, tau is distributed equally between cell body and neurites. When the axon emerges and the neuron is polarized, tau becomes enriched in the axon and to a lesser extent, in dendrites and the nucleus. The process that underlies polarized distribution in mature neu-

Abbreviations: *MAPT*, Microtubule associated protein tau; UTR, untranslated region; CDS, coding DNA sequence; mRNA, messenger RNA; mTOR, mammalian target of rapamycin; 5'TOP, 5' terminal oligopyrimidine; RIN, RNA integrity number.

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rons remains poorly understood, as it takes place at both mRNA and protein levels [3]. *MAPT* mRNA is distributed throughout the proximal axon due to an axon localization signal in the 3'UTR region [4]. Tau is also preferentially translated in axons, which is dependent on mTOR (mammalian target of rapamycin) signaling [5]. The *MAPT* 5'UTR has a 5' terminal oligopyrimidine (5'TOP) track that mediates mTOR-governed protein synthesis [6]. Differential distribution of tau and its isoforms allows diverse functions [7]. The *MAPT* 3'UTR binds several proteins, such as HuD, IMP-1, G3BP, Ilf3, and NF90, that are potentially associated to *MAPT* mRNA axonal localization [8,9]. Perturbation of *MAPT* mRNA sorting and distribution might induce toxic gain of function and contribute to tauopathies [10].

MAPT 3'UTR also has important cis-elements that decrease its RNA expression [7], as well as several micro-RNA binding sites such as miR219, miR34a, miR35c-5p, miR485-5p and miR132-3p; this last is strongly downregulated in Alzheimer [11–15].

In this study, we show that several genes are unbalanced in Alzheimer pathology. This misregulation involves notable loss of neuronal markers, in contrast to increased glial hallmarks and unaffected microglial and oligodendroglial markers. We observed that the neuronal marker *MAPT* mRNA is reduced, whereas tau protein is increased. In addition, the ratio of 3'UTR to the CDS, which appears to be balanced in human and mouse normal brain tissue, was significantly increased in Alzheimer disease. Our data indicate that the amount of tau protein does not correlate with the mRNA levels, and highlight the importance of non-coding regions in the regulation of protein synthesis.

2. Materials and methods

2.1. RNA-seq data analysis

RNA-seq data from mouse and human brain samples were downloaded from the NCBI Sequence Read Archive (SRA) repository using the SRP053398 and SRP004879 identifiers, respectively. SRP053398 contains 8 mRNA samples from brain striatum of WT q175 mice [3] that were analyzed with Illumina HiSeq 2000, and paired-end reads of 50 bases. SRP004879 contains 3 pooled mRNA samples from normal human brain (whole brain, frontal lobe, and temporal lobe) and 3 mRNA samples from Alzheimer patients (whole brain, frontal lobe, and temporal lobe; [16]) (see Table 1), analyzed with Illumina Genome Analyzer II, with single-end reads of 36 bases. Raw data were downloaded as SRA files, converted to FASTQ files using the NCBI SRA-Toolkit (v. 2.6.2), and mapped to genomes using TopHat2 (v. 2.1.1) [17]. We used transcript annotations files gencode.v24.annotation.gtf (human) and gencode.vM4.annotation.gtf (mouse) (both obtained from the GENCODE website, <http://www.genencodegenes.org>). Genome versions GRCh38.77 (human) and GRCm38.76 (mouse) were used, obtained from the ENSEMBL website (<http://www.ensembl.org/index.html>). Read depth from specific exons and 3'UTR regions were obtained directly using the output BAM files from TopHat2 using SAMtools (v. 1.3.1) [18]. Briefly, read depth was calculated for the last three exons and the 3'UTR region for human and mouse samples. CDS expression was calculated as the average read depth of the three exons, thus ruling out differential coverage between 5' and 3' regions within mRNAs due to retrotranscription using oligo-dT before massive sequencing.

2.2. Brain tissue

Hippocampal brain samples from sporadic Alzheimer disease patients and control subjects were obtained from the Banco de Tejidos (Fundación CIEN, Instituto de Salud Carlos III, Madrid, Spain). Based on quantitative pathological features, the Alzheimer brain

specimens ($n = 16$) were classified according Braak stages II ($n = 3$), III ($n = 2$), V ($n = 7$) and VI ($n = 4$), and non-demented control subjects ($n = 8$) (see Supplementary Table S1 in the online version at DOI: [10.1016/j.neulet.2017.07.007](https://doi.org/10.1016/j.neulet.2017.07.007) for further subject information). Written informed consent was obtained from all patients.

2.3. Western blot analyses

Frozen brain tissue was homogenized using a TissueLyser (Retsch MM300, Qiagen, Hilden, Germany) (30 Hz, 5 min) with 5-mm stainless steel beads (69989, Qiagen) in 50 mM Tris-HCl pH 7.5, 300 mM NaCl, 0.5% SDS (sodium dodecyl sulphate) and 1% Triton X-100. The homogenate was incubated (15 min, 95 °C), centrifuged ($16,100 \times g$, 10 min) and supernatant considered brain extract. Protein concentration was measured using the Dc protein assay kit (500-0111, Bio-Rad). From each sample, 30 μ g total protein were resolved on a 10% Bis-Tris gel and transferred to PVDF membranes. Blots were probed with an anti-tau antibody (anti-body 7.51) [19], followed by horseradish peroxidase-conjugated anti-mouse antibody (Dako, Glostrup, Denmark). Tau expression was quantified by measuring the ECL signal per lane with ImageJ software (<http://rsbweb.nih.gov/ij/>).

2.4. Microarray analysis

The expression profiling by array (Affymetrix Human Gene 1.0 ST Array) was retrieved from the Hisayama study (GEO id: 4758) [20] for following genes in human brain samples classified as healthy ($n = 47$) vs. Alzheimer disease ($n = 32$): *MAPT*, *MAP2*, *TUBB3*, *NeuN*, *Syt1*, *Grba1* and *Snap25* as neuronal markers; *GFAP*, *S100B*, *Adhl11l* and *Aqp4* as astrocytic markers; *IBA1*, *CD68*, *CD45* and *CD11b* as microglial markers; *GalC*, *Mog*, *SOX10* and *Cx47* as oligodendrocytic markers [21]. Differences in expression of each gene between healthy and Alzheimer samples were calculated using Student's *t*-test.

2.5. Quantitative real-time PCR assays

Frozen brain tissue was homogenized using a TissueLyser in conditions as above, in 700 μ l QIAzol Lysis Reagent (79306, Qiagen). RNA was extracted using miRNeasy Mini Kit (217004, Qiagen) with RNase-Free DNase Set (79254, Qiagen) following manufacturer's guidelines. The RNA integrity number (RIN) of each sample was calculated using the Agilent 2100 Bioanalyzer system (Agilent Technologies), and only RNAs with RIN >5 were used for RT-qPCR. RNA was retrotranscribed with the Transcriptor First Strand cDNA Synthesis Kit (04379012001, Roche) using 20 ng/ μ l RNA with random primers. Quantitative PCR was performed in a LightCycler480 (Roche) in the following conditions: 50 °C for 2 min, 95 °C for 10 min, and 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Oligonucleotides used for detection of *MAPT*-CDS: *MAPT*-E11-E13-fw GTCGAAGATTGG GTCCCTGG and *MAPT*-E11-E13-rv GACACCACTGGCGACTTGTA, for *MAPT*-3'UTR: *MAPT*-3UTR-B-fw TTTTGGCCACTTTGCAGACC and *MAPT*-3UTR-B-rv TCTCCAA GAGGCACAAGTC, and for *TUBB3*: *TUBB3*-fw AACGAGGCCTCTTCTCACA and *TUBB3*-rv GGCCTGAA-GAGATGTCCAAA. Gene expression was normalized to GAPDH expression using TaqMan primer human GAPDH (Hs02758991.g1, Applied Biosystems).

2.6. Statistical analysis

Quantitative data, represented as mean $\pm$ SD or SEM were compared between groups using the two-tailed Student's *t*-test.

Table 1
Source of total RNA from brain tissue samples.

condition	sample	gender	Age (years)	Source
normal	Whole brain	13 male; 10 female	23–86	Ambion
	Frontal lobe	5 male	22–29	Capital Biosciences
	Temporal lobe	5 male	23–29	Capital Biosciences
Alzheimer's disease	Whole brain	1 male	87	Capital Biosciences
	Frontal lobe	1 male	87	Capital Biosciences
	Temporal lobe	1 male	80	Capital Biosciences

<http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0016266>.

Differences are presented with *P* value (* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; N.S. not significant).

3. Results

3.1. MAPT mRNA 3'UTR-to-CDS ratio is balanced in human and mouse brain

Map2 has relatively similar 3'UTR-to-CDS expression in dopaminergic neurons as seen in RNA-seq analysis; some mouse brain regions nonetheless show unbalanced 3'UTR-to-CDS expression [22]. Cells with a 3' UTR-to-CDS ratio >3 do not express Map2 [22]. We studied this ratio for MAPT in published RNA-seq samples from mouse brain striatum [23], and found a balanced 3'UTR-to-CDS ratio in the eight wild-type mice (Fig. 1A and B). In a similar assay using reported human brain RNA-seq data [16] from whole brain and temporal and frontal lobes of healthy individuals (Fig. 1C, see Table 1), we found moderate imbalance, with slightly higher amounts of 3'UTR compared to CDS in all samples (Fig. 1D).

3.2. MAPT mRNA is reduced in Alzheimer disease patients

Tau protein accumulates in Alzheimer disease brain [24], and advance of the pathology correlates with this accumulation [25]. Our collection of hippocampal brain samples showed a strong increase in tau protein levels in Alzheimer patients ($n=16$) vs. non-demented controls ($n=8$) (Fig. 2A, Supplementary Table S1 in the online version at DOI: 10.1016/j.neulet.2017.07.007). Microarray data from control ($n=47$) and Alzheimer disease brain samples ($n=32$) from the Hisayama study [20] nonetheless showed significantly lower MAPT mRNA levels in Alzheimer vs. non-demented brain (Fig. 2B, Table 2). Given this apparent contradiction, we hypothesized that the proportion of neurons in Alzheimer brains was reduced as a result of neuronal degeneration.

To test this hypothesis, we analyzed several neuronal markers [26] in this sample set and found that neuronal structural genes such as MAP2 or TUBB3 (β III-tubulin), nuclear protein genes such as NeuN, and other neuronal markers were significantly lower in Alzheimer vs. control brain (Fig. 2B, Table 2). Analysis of markers for other cell components of the brain indicated that all astrocyte-associated markers were increased in Alzheimer brain (Fig. 2C), whereas those for microglia (Fig. 2D) and oligodendrocytes (Fig. 2E) showed no marked differences (Table 2). We thus identified a clear imbalance between tau protein and mRNA levels in Alzheimer patient brains.

3.3. MAPT RNA 3'UTR-to-CDS ratio is increased in Alzheimer disease

Although MAPT 3'UTR was only moderately enriched in human brain samples, we sought to determine its functional relevance. Given the importance of tau in Alzheimer pathology, we studied the 3'UTR-to-CDS ratio in patient brain mRNA samples (Fig. 3A, Table 1). Differences between MAPT 3'UTR and CDS were increased

in Alzheimer samples, in which the number of 3'UTR reads doubled those of the CDS region, with a ≥ 2 -fold increase (Fig. 3B). We validated this result in a RT-qPCR assay in hippocampal brain samples from non-demented controls ($n=8$) and Alzheimer patients ($n=16$) (Supplementary Table S1 in the online version at DOI: 10.1016/j.neulet.2017.07.007), using random primers for retrotranscription rather than oligo-dT, to avoid enrichment in 3' poly-A regions. Results showed significant differences in 3'UTR levels between non-demented and Alzheimer samples (Fig. 3C). There was a slight decrease in MAPT CDS levels in Alzheimer brain samples, in which we found high tau levels (Fig. 1A). This reduction might be related to the decreased neuron numbers and increased gliosis in these patients [27]; indeed, mRNA levels for the neuronal marker TUBB3 was significantly reduced in Alzheimer samples (Fig. 3C). To confirm this idea, we studied the ratio of MAPT CDS and 3'UTR normalized to neuron number as estimated by TUBB3 expression. Although MAPT CDS was similar in non-demented and Alzheimer patients (Fig. 3D and E), the MAPT 3'UTR was greatly increased in Alzheimer patient brains when normalized to neuron number (Fig. 3D). In addition, the MAPT 3'UTR-to-CDS ratio in these samples was significantly increased in Alzheimer relative to non-demented control brain (Fig. 3F).

4. Discussion

Studies in neurons show an unbalanced 3'UTR-to-CDS ratio for some mRNAs. As this imbalance might correlate with variations in protein expression involving several neuronal markers including some MAP [22], we sought to characterize MAPT mRNA stoichiometry, focusing on 3'UTR abundance relative to CDS. We hypothesized that variations in Alzheimer patient MAPT 3'UTR correlate with changes in tau protein levels, which would offer an additional mechanism to explain the tau pathology associated with the disease.

Physiological and pathological expression of the MAPT/tau gene shows complex regulation of both mRNA expression and localization [7]. We demonstrated reduced MAPT mRNA expression in Alzheimer brain, which we explain as a result of neuron loss in these patients, as MAPT levels relative to the constitutive neuronal marker TUBB3 were unchanged in healthy and diseased tissue. An earlier study reported increased MAPT mRNA levels in hippocampus of Alzheimer disease and other dementia patients [28]. Here we report that quantitative PCR, microarray and RNA-seq analyses indicated reduced MAPT mRNA levels. The difference between the two studies could be due to distinct experimental approaches. The work by Barton et al. used *in situ* hybridization to detect MAPT RNA, with a probe located at the beginning of the CDS and normalized to total poly-A RNA [28]. In our qPCR analysis, RNA integrity was confirmed for each sample; in addition, we used intron-spanning oligonucleotides that rule out genomic DNA contamination and are directed against the end of the CDS and the beginning of the 3'UTR. Our data were normalized to GAPDH mRNA, which was tested for stability in our sample set. In addition, we validated our

Fig. 1. RNA-seq analysis shows a balanced *MAPT* 3'UTR-to-CDS ratio.

A. RNA-seq profile of q175 mice ($n = 8$) showing the last 3 exons and 3'UTR of *MAPT* mRNA. B. Graph of the ratio of 3'UTR read depth relative to CDS (mean of last 3 exons) of the mice. C. RNA-seq of *MAPT* showing last 3 exons and 3'UTR of human healthy pooled mRNA brain samples ($n = 3$, whole brain, temporal lobe, frontal lobe) (see Table 1). D. *MAPT* 3'UTR-to-CDS ratio of samples in C.

qPCR results by microarray and RNA-seq analysis, both accurate quantitative techniques.

On the other hand, whole-exome sequencing of 522 early-onset cases and 584 controls showed that <1% of Alzheimer patients (4/522) had microduplications in chromosome 17 at the *MAPT* locus [29]. This analysis showed that this rare copy number variation in Alzheimer patients, which correlated with significantly increased *MAPT* mRNA levels in their blood cells. It could be of interest to explore *MAPT* mRNA levels in the brains of patients that harbor this duplication. The fact that an increased *MAPT* mRNA level correlated with Alzheimer pathology in a small percentage of cases contrasts with our finding of decreased mRNA levels, and indicates

that an RNA stoichiometric imbalance could have a fundamental role in the disease. Further studies are needed to fully understand the involvement of RNA levels in this pathology.

We observed an inverse correlation between *MAPT* mRNA and tau levels. Another report described correlation between tau and *MAPT* mRNA levels during development [30]. However, *MAPT* mRNA can be transcribed in cells several days before tau protein is actively translated [31], which suggests that factors other than mRNA levels might determine activation of translation to protein. High tau levels when mRNA is decreased might indicate a protein degradation defect. The principal tau degradation pathways are ubiquitin-proteasome and autophagy-lysosome systems [13].

Fig. 2. Unbalanced expression of tau and neuronal markers in Alzheimer brain.

A. Quantification of tau protein expression measured by Western blot (not shown) of non-demented control ($n = 8$) or Alzheimer disease ($n = 16$) hippocampal brain samples. Graph shows mean $\pm$ SEM for each lane of the Western blot, for which 30 μ g of each brain sample were resolved. Significance was determined by Student's t -test, ** $p < 0.01$. B–E. Analysis of gene microarray expression for neuron (B), astrocyte (C), microglia (D) and oligodendrocyte (E) markers. Graphs show dot plots of representative marker expression in $n = 47$ controls and $n = 32$ Alzheimer disease brain samples retrieved from the Hisayama study [20]. Data shown as means $\pm$ SD; significance was determined by Student's t -test and is shown for each graph.

Proteasome degradation is diminished in Alzheimer disease patient brain [32], and posttranslational modifications of tau such as phosphorylation or aggregation might inhibit proteasome degradation [33]. Alterations in the autophagy pathway have been demonstrated in the disease [34]; tau accumulation might thus be a result of deficient degradation rather than an increase in its synthesis. Moreover, mTORC1 signaling is increased in Alzheimer brain [11] and inhibits autophagy [35], thus contributing to tau accumulation. mTORC1 activation induces *MAPT* mRNA translation into protein

by recognition of the 5'TOP motif in its 5'UTR [6], another possible mechanism of tau protein increase. Finally, not only tau levels but also its posttranslational modifications, including phosphorylation, glycosylation, glycation, prolyl-isomerization, cleavage or truncation, nitration, polyamination, ubiquitination, sumoylation, oxidation and aggregation, might have a fundamental role in Alzheimer pathogenesis [36]. Further study is needed to determine how *MAPT* 3'UTR levels correlate with these modifications in addition to the protein increase.

Fig. 3. *MAPT* 3'UTR-to-CDS ratio is increased in Alzheimer disease.

A. RNA-seq of *MAPT* showing the last 3 exons and 3'UTR of Alzheimer ($n = 3$) samples from whole brain, temporal lobe and frontal lobe (see Table 1). B. *MAPT* 3'UTR-to-CDS ratio of samples in A. C. Relative mRNA levels measured by RT-qPCR of the 3'UTR or the coding region (CDS) of *MAPT*, as well as β III-tubulin (*TUBB3*) of human hippocampus (non-demented controls $n = 8$, Alzheimer disease $n = 16$). D. *MAPT* CDS and 3'UTR ratios relative to *TUBB3* of the same sample set. E. Analysis of gene microarray expression of *MAPT* relative to *TUBB3* in controls ($n = 47$) and Alzheimer disease ($n = 32$) brain samples retrieved from the Hisayama study [20]. F. *MAPT* 3'UTR-to-CDS ratio of human hippocampus from the sample set in C. Data shown as mean $\pm$ SEM (C, D and F) or mean $\pm$ SD (E); p value determined by Student's t -test. ** $p < 0.01$, *** $p < 0.001$, N.S. = not significant.

Table 2
Changes in expression levels of markers in Alzheimer brain using gene microarray.

Neuronal markers							
GENE	MAPT	MAP2	TUBB3	NeuN	Syt1	Grba1	Snap25
AD vs. control	↓ $p = 0.01$	↓ $p = 2 \times 10^{-5}$	↓ $p = 0.002$	↓ $p = 0.0002$	↓ $p = 0.001$	↓ $p = 0.0002$	↓ $p = 9 \times 10^{-5}$
Astrocytic markers							
Gene	GFAP	S100B	Aldh111	Aqp4			
AD vs. control	↑ $p = 0.0005$	↑ $p = 0.05$	↑ $p = 0.007$	↑ $p = 0.02$			
Microglial markers							
Gene	IBA1	CD68	CD45	CD11b			
AD vs. control	N.S.	N.S.	N.S.	↑ $p = 0.04$			
Oligodendrocyte markers							
Gene	GalC	Mog	SOX10	CX47	MBP		
AD vs. control	N.S.	N.S.	N.S.	N.S.	N.S.		

The 5'UTR region is involved in ribosome recruitment to mRNA and start codon choice [37]; less is known about the 3'UTR region. The *MAPT* 3'UTR region was characterized and was implicated in *MAPT* mRNA stabilization in neuronal cells [4,38]. This region contains AU-rich elements (AREs) that could direct poly-A tail addition to the end of *MAPT* mRNA.

Different amounts are reported of coding region (exons) and 3'UTR sequences in several neuronal genes [22]. Some genes have a 3'UTR without a CDS [39], although it remains to be clarified whether this results from expression of a RNA from 3'UTR, or to distinct degradation of CDS and 3'UTR regions. In addition, a high 3'UTR-to-CDS ratio can lead to a decrease in protein levels [22].

We observed a balanced 3'UTR-to-CDS *MAPT* ratio in normal mouse and human tissue, similar to that described for *Map2* [22]. In individual cells, however, these authors found that a high 3'UTR-to-CDS ratio coincided with reduced protein expression. We found an increased *MAPT* mRNA 3'UTR-to-CDS ratio in Alzheimer that coincides with increased tau levels, in contrasts those findings for *Map2*. A question that arises from these data is whether the increased 3'UTR-to-CDS *MAPT* ratio promotes mTORC1-dependent translation and thus allows the aberrant tau expression observed in Alzheimer brain. This question must be explored, taking into consideration the complexity of *MAPT* 3'UTR, not only due to its different-length isoforms but also to the many proteins that bind this mRNA region.

5. Conclusions

Here we describe an inverse correlation in *MAPT* mRNA and tau protein levels, and an increased 3'UTR-to-CDS ratio in Alzheimer patient brain compared to non-demented controls. Reduction of the tau levels could be beneficial for disorders like Alzheimer, in which intracellular tau accumulation could be toxic to neurons [26,40–42], and induce synaptic impairment and memory deficits in mice [43,44]. Understanding the regulation of tau translation and the potential involvement of non-coding RNA regions could be a clue for treatment of such disorders. Our results suggest that disease models in which complete mRNA is used, including 5' and 3'UTR, are most appropriate for the study of Alzheimer disease, as they might be key regulatory elements in tau protein expression.

Conflict of interest

All authors declare that they have no conflict of interest.

Author contributions

G.E, R.G, P.M.M, E.G. and R.G.E performed the experiments. V.G.E, R.G. and R.G.E. designed the experiments and analyzed the data. V.G.E, R.G., R.G.E and J.A. wrote the manuscript, and provided editorial suggestions and criticisms.

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