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# MiR-107 is reduced in Alzheimer's disease brain neocortex: validation study

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## Abstract

MiR-107 is a microRNA (miRNA) that has been shown to have decreased expression in the temporal cortical gray matter of humans early in the progression of Alzheimer's disease (AD). Here we study a new group of well-characterized brain samples (N=19). MiR-107 expression was assessed, normalized to other miRNAs. Quantitative AD histopathology was performed on adjacent tissue. Statistical regression analyses were performed comparing neuritic plaque (NP) and neurofibrillary tangle (NFT) counts. Correlation was observed between decreased miR-107 expression and NP counts ($P<0.05$), and NFT counts ($P<0.02$). Among these samples adjusted miR-107 and BACE1 mRNA levels tend to be correlated negatively as detected with qPCR (trend with regression $P<0.07$). In sum, miR-107 expression tended to be lower relative to other miRNAs as AD progresses.

## Introduction

MicroRNAs (miRNAs) are short (~22 nucleotides) non-coding regulatory RNAs that serve vital functions in the mammalian brain. For example, miRNAs are important players in neurodevelopment and synapse function [1,2]. Moreover, miRNAs also participate in (thus far mostly unknown) neuroprotective functions because after miRNA depletion neurons tend to degenerate relatively quickly [3–6].

Aside from their relevance to basic neurobiological processes, miRNAs may also contribute to specific neurodegenerative diseases. Aberrant miRNA biology has been implicated in Alzheimer's disease (AD) [7–13]. However, this field is still in its infancy and the initial findings need to be refined, replicated, and better understood.

We previously described that the expression of a particular miRNA (miR-107) becomes downregulated in parallel with the progression of AD in human cerebral cortical gray matter [11]. This led to a specific hypothesis about how miRNAs can contribute to AD pathogenesis because miR-107 targets the beta-site amyloid precursor protein catalyzing enzyme 1 (BACE1) which is upregulated in AD brains [11].

Although miRNAs have been studied in AD brains, there has been limited validation of prior results. Here we sought to analyze samples from the University of Kentucky Alzheimer's Disease Center (UK ADC) autopsy series to either validate or refute the earlier finding that miR-107 is altered in the course of AD. AD is defined neuropathologically by the presence of neurofibrillary tangles (NFTs) and neuritic plaques (NPs) [14]. Thus we

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assessed the level of miR-107 in the brain in correlation with NFT and NP counts using biochemical methods and histopathology in adjacent tissue sections.

## Materials and Methods

### RNA isolation from a human cerebral cortex

All analyses were performed blind with respect to patient information. Cases were selected on the bases of representing the full spectrum of clinical/pathological progression of AD, and of their being available more recently than our prior study [15] on miRNAs in AD brain. RNA was extracted from snap-frozen brain tissue in the superior and middle temporal gyri (SMT; Brodmann Areas 21/22) from the UK ADC under a University of Kentucky IRB protocol. Premortem clinical evaluations and pathological assessments were as described previously [15–17]. The inclusion criteria that were applied: post-mortem interval (PMI) <4hrs; no argyrophilic grains; no cortical Lewy bodies (LB); no evidence of frontotemporal dementia; no cancer in the brain parenchyma; and no large infarctions in the brain, or microinfarcts found within 3cm of the brain tissue samples. These neuropathological confounds were assessed using standard neuropathological procedures as described in detail elsewhere [16,18]. NFTs and NPs were counted in the SMT as described previously in detail [16]. Included patient characteristics, along with pathological lesion counts in the same cases, are shown on Table 1.

RNA was isolated as previously described in detail [11,15]. Briefly, Prior to RNA extraction, gray matter was dissected away from white matter and only gray matter was used for these studies. RNA was isolated from brain tissue cut from superior and middle temporal cortex. Adjacent tissue was evaluated neuropathologically for all samples. Tissue (1–3 gms) that had been snap-frozen in liquid nitrogen and then transferred to a −80°C freezer was thawed in isotonic lysis buffer with RNAsin (Promega, Madison, WI; 250 U/ml) and Complete protease inhibitor pills (Roche, Basel Switzerland). Trizol LS (Invitrogen, Carlsbad, CA) was used according to manufacturer's instructions, except for an additional overnight −20°C precipitation step during isopropanol precipitation. RNA quality was confirmed using A260/A280 readings.

### RTqPCR and expression analyses

As with the RNA isolation, all steps prior to final data analyses were performed blindly in regard to clinical and pathological information. For qPCR analysis of miRNAs, total RNAs were subjected to RT-qPCR using miRNA detection kits (Ambion-Applied Biosystems, Foster City, CA). qPCR was performed using ABI Prism 7000 Sequence Detection System (Applied Biosystems, Foster City, CA) with separate standard curves for each miRNA. Three separate qPCR runs (with three replicates each) were performed on all the data and the data represent averaged values of the qPCR readouts. For qPCR analysis of BACE1 and β-Actin, RNAs were reverse-transcribed with qScript cDNA SuperMix (Quanta Biosciences, Gaithersburg, MD), amplified using TaqMan 2X PCR Master Mix (Applied Biosystems, Branchburg, NJ) and SYBR as detector. BACE1 levels were normalized to β-Actin levels.

MiR-107 expression, as indicated by qPCR, was normalized to the expressions of miR-124 and let-7a in the same cases. These control miRNAs were selected because we evaluated them previously in AD and nondemented brains and they were not altered in AD brain [11]. No other miRNA levels were evaluated.

## Results

Levels of miR-107 (normalized to miR-124 and let-7a) were assessed and correlated with the amount of AD-type pathology in adjacent tissue sections. This approach was utilized

rather than correlating solely with Braak Staging [19] of NFTs or Consortium to Establish a Registry for Alzheimer's Disease scores of NP densities because the latter pathological rating scales provide ordinal rather than continuous variables for regression analyses. Linear regression data correlating the amount of AD-type pathology with the normalized miR-107 expression (Figure 1) indicated that miR-107 expression tended to correlate in a negative fashion with NPs ($P<0.07$) and NFTs ($P<0.04$). By contrast, miR-107 expression did not correlate in any way with age of death or PMI. When assessing the correlation between miR-107 expression and NP density in these human neocortical samples, the R-squared correlation coefficient was 0.19 (Figure 1). Note that there are several cases with relative low miR-107 levels despite also having low amount of AD-type pathology in adjacent tissue (Arrow in Figure 1).

## Discussion

Brain samples from the UK ADC autopsy series were used as a validation cohort to test how miR-107 expression correlates with AD pathological markers. Gray matter tissue from the SMT was used and RNA isolated as described previously. MiRNAs were quantified using qPCR. This study supports the prior observation miR-107 expression decreases relative to other miRNAs as AD progresses[11].

There are limitations to the current study. The results are circumscribed – thus providing only an incremental gain of information – and pertain to the measurement of a few miRNAs in human brain. It has been shown previously that miRNA levels can be artifactually (and unpredictably) changed in the human brain postmortem [20]. In this study we used short PMI cases and did not see an effect in miRNAs that could be correlated with PMI. Nor was PMI different in high-pathology versus low-pathology cases. However, there is a possibility that unanticipated biases related to differential miRNA stability could affect our results.

Although we find in this validation cohort that miR-107 levels in brain tend to decrease in correlation to increased AD pathology, we also find (as in the prior study) that there is considerable overlap in miR-107 levels across the gamut of cases. For example, as shown in Figure 1, miR-107 levels are still relatively low in two brains that lacked appreciable AD pathology. In any case it is unknown how miR-107 levels can be determined in cerebrospinal fluid for in vivo analyses. These considerations are important because there have been suggestions of using miRNA levels as biomarkers for neurodegenerative diseases including AD [7]. MiR-107 expression in this portion of the brain does not appear to be a good candidate biomarker to differentiate the diseased from non-diseased brain.

If there is a trend for miR-107 to decrease in AD brain, what does it mean? We have previously identified a specific target of miR-107 (BACE1) that could provide links to AD pathogenesis [11]. This raises questions about biological factors upstream to miR-107 gene expression regulation. Evidence points to the roles of miR-107 in cellular metabolism [21,22]. Levels of miR-107 expression in cultured cells are relatively high in high-glucose or low folate culture media conditions [21,23]. In all known vertebrates, the genes for miR-107 and miR-103 reside within introns of pantothenate kinase genes. Pantothenate kinase polypeptides catalyze the enzymatic rate-determining step in Acetyl-CoA formation [22]. It is tempting to speculate that the miR-107 story may help link the known metabolic dysfunction seen very early in AD brains [24,25] with the development of pathology in the disease course.

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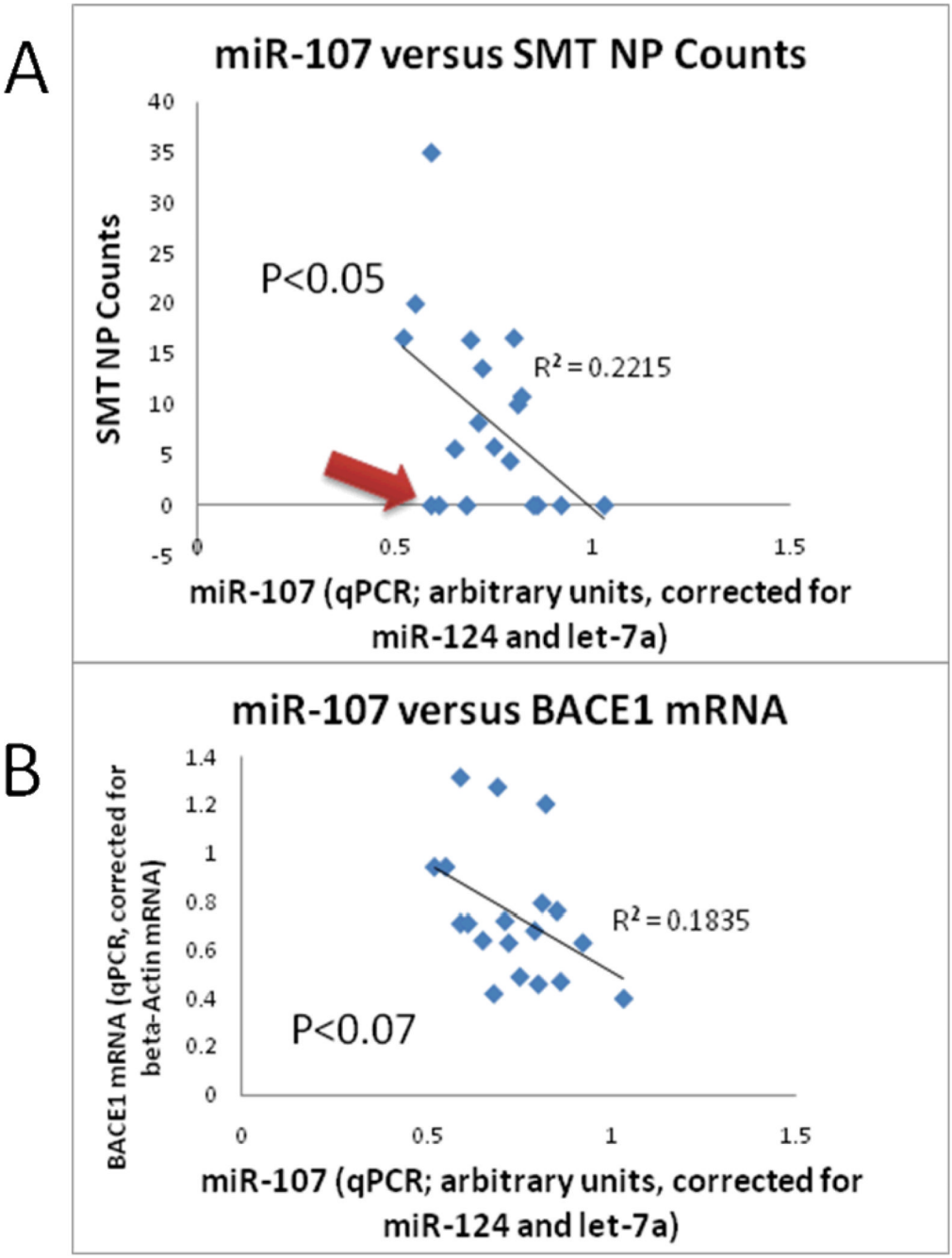


**Figure 1.**

Charts shows the results of 19 cases evaluated, correlating the normalized expression of miR-107 with neuritic plaque lesion density counts in adjacent tissue sections from superior-mid temporal cortical (SMT) gyri (A) and BACE1 mRNA levels (B). There is a trend for decreased miR-107 in cases with more neuritic plaques (regression $P<0.05$). Note, however, that there were cases (red arrow) with low miR-107 expression despite lacking any SMT neuritic plaques. The negative correlation between miR-107 and BACE1 mRNA levels does not reach statistical significance ($P<0.07$).

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**Table 1**

Demographic and pathologic information along with RNA data on patient cohort.

| Case | Braak stage (0-VI) | Age | Sex | PMI (hrs) | Brain Weight (gms) | SMT NP Counts | SMT NFT Counts | ApoE Alleles | miR-107 (normalized arbitrary units) | BACE1 mRNA (normalized arbitrary units) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | I | 75 | F | 3.50 | 1330 | 0 | 0 | 3/3 | 0.59 | 0.71 |
| 2 | I | 100 | F | 2.25 | 1320 | 0 | 0.2 | 2/3 | 0.61 | 0.71 |
| 3 | I | 84 | F | 3.00 | 900 | 0 | 0 | 3/3 | 0.86 | 0.47 |
| 4 | I | 84 | F | 2.50 | 1100 | 0 | 0 | 3/3 | 0.92 | 0.63 |
| 5 | IV | 96 | F | 2.50 | 970 | 0 | 1 | 3/3 | 0.68 | 0.42 |
| 6 | II | 92 | F | 3.00 | 1010 | 0 | 0 | 3/3 | 1.03 | 0.40 |
| 7 | IV | 78 | M | 1.20 | 1130 | 0 | 0 | 3/3 | 0.85 | 0.76 |
| 8 | IV | 91 | M | 2.00 | 1210 | 4.4 | 2.4 | 3/4 | 0.79 | 0.68 |
| 9 | IV | 84 | M | 3.50 | 1350 | 5.6 | 1.2 | 3/4 | 0.65 | 0.64 |
| 10 | III | 86 | F | 3.25 | 1130 | 5.8 | 0 | NA | 0.75 | 0.49 |
| 11 | III | 92 | F | 3.25 | 1220 | 8.2 | 1.2 | 2/3 | 0.71 | 0.72 |
| 12 | 0 | 92 | M | 3.33 | 1220 | 10 | 0 | 3/3 | 0.81 | 0.79 |
| 13 | V | 94 | F | 3.00 | 1070 | 10.8 | 15.8 | 3/3 | 0.82 | 1.20 |
| 14 | V | 94 | M | 2.00 | 1110 | 13.6 | 12.6 | 3/3 | 0.72 | 0.63 |
| 15 | III | 88 | M | 3.00 | 1130 | 16.4 | 0 | 3/3 | 0.69 | 1.27 |
| 16 | VI | 86 | M | 3.25 | 1150 | 16.6 | 41.4 | 3/4 | 0.52 | 0.94 |
| 17 | IV | 77 | M | 2.75 | 1340 | 16.6 | 5.6 | 3/4 | 0.80 | 0.46 |
| 18 | VI | 99 | F | 2.10 | 1150 | 20 | 23.6 | 3/4 | 0.55 | 0.94 |
| 19 | VI | 73 | M | 2.00 | 1130 | 35 | 42.6 | 3/3 | 0.59 | 1.31 |

19 cases were used in the study, arranged from lowest to highest in terms of neuritic plaque density. RNAs are quantified with qPCR--miR-107 is normalized to miR-124 and let-7a. BACE1 mRNA levels were normalized to beta-Actin mRNA. PMI=post-mortem interval. Brain weight is in grams. SMT=superior and mid-temporal cortical gyri. NP = Neuritic plaque. NFT = Neurofibrillary tangle. “Counts” refer to lesion densities (average of five highest 20× fields). N/A=Not available.