

PERSPECTIVE

The paradox of opposite directions of gene expressions in MCI and AD suggests possible therapy to prevent progression of MCI to AD

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Abstract

One of the puzzling observations concerning mild cognitive impairment (MCI) and Alzheimer's disease (AD), is that many gene expressions in MCI may be in the opposite direction of those seen in AD. Several examples of this paradox are provided. The likely explanation lies in the control mechanisms of gene transcription. These mechanisms include (1) modification of DNA and histones by methylation or acetylation, affecting the balance between the Compass group of proteins that enhances mRNA formation, and the Polycomb group that suppresses it; (2) compensation for the loss of one gene's function by another gene with overlapping functions; (3) reduced control of the entire neural RNA production; and (4) response to microRNAs (miRNA). Although data are inadequate to exclude with certainty any one of the indicated mechanisms, the available evidence favors overall reduced control of neural mRNA production, including the effect of miRNA. The switch occurs at a specific stage, somewhere between Braak 0-1 and Braak 2-3, in the progression from MCI to AD, which reduces the number of its likely causes. Two strong but related candidates are the repressor element-1 silencing transcription factor (REST), which in adult neurons impairs plasticity; and a miRNA, for example, miRNA124, that represses REST. Another possible explanation is that only those patients with MCI who will not progress to AD are the ones that have gene expressions in the opposite direction as in AD. The solution to the paradox may have pragmatic value.

1 | INTRODUCTION

If mild cognitive impairment (MCI) is the forerunner of Alzheimer's disease (AD), as is often the case, surely any aberrancies of biochemical pathways in MCI should resemble, even if to a lesser degree, those found in AD. However, among the puzzling observations that have been reported for MCI and AD, one that ranks highly is that many gene

expressions in MCI may be in the opposite direction of those seen in AD. Offered below is a possible explanation for this apparent paradox. If the explanation is correct, then identifying its biochemical underpinning has heuristic value. First will be a brief description of the disparate findings of gene expressions in MCI and AD; next will be a tentative explanation; finally is an indication of possible approaches to treatment that, assuming the suggested explanation is correct, might prevent the

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switched direction of gene expressions between MCI and AD and, thus, stop progression from MCI to AD.

2 | IN AD, GENE EXPRESSION IS MOSTLY DOWNREGULATED

In AD, downregulation of genes dominates upregulation. Focusing on the CA1, one study correlated the expressions of 12,665 genes with both MMSE scores and counts of neurofibrillary tangles (NFT), and found 898 genes over-represented in the AD group, including 35% more downregulated than upregulated ones.¹

Examination of the hippocampus for genes controlling synaptic function (synaptic vesicle trafficking and release; neurotransmitter receptors and receptor trafficking; postsynaptic scaffolding and cell adhesion), and for genes controlling brain derived neurotrophic factor (BDNF), showed that AD patients had twice the number of expression changes, all of them downregulated, among synaptic genes as in controls, and expression of BDNF declined to as little as 20% of the level seen in young controls.² Another report showed that in the CA1 of an AD brain, nine of the ten genes affecting synaptic function were downregulated.³

However, in a different study, less difference in gene expressions than the above was seen in the hippocampi of AD, where 372 genes were either upregulated or downregulated.⁴ The largest number of genes relevant to AD were those linked to synaptic function and neuronal plasticity (21 downregulated and 21 upregulated), to signaling of calcium (14 downregulated and 14 upregulated), to phosphatidylinositol (7 downregulated and 13 upregulated), to insulin (6 downregulated and 13 upregulated), and to Wnt (6 downregulated and 8 upregulated). Variation in another analysis depended upon the brain region as well as the identity of genes examined. In that study, gene expressions known to be associated with either amyloid plaques or NFT showed, in the entorhinal cortex, downregulation in 59%, upregulation in 15%, and indeterminate regulation in 26%; in the hippocampus, it showed downregulation in 30%, upregulation in 35%, and indeterminate regulation in 35%.⁵ Considering the gene expressions associated with NFT regions separately from those associated with amyloid showed, in the entorhinal cortex, downregulation in 70%, upregulation in 12%, and indeterminate regulation in 18%; in the hippocampus, it showed downregulation in 47%, upregulation in 18%, and indeterminate regulation in 35%. In regard to whether changed gene expressions have good, bad, or indifferent implications, it is worth noting their effects. For example, in CA1 of AD brains, DAX, FAS, and DPP1, which mediate apoptosis, were upregulated 3.8- to 4.8-fold, and genes expressing IL-1 β , IL-1 α , and IL-6, were upregulated ≥ 3.0 -fold; in the same study, genes expressing synaptophysin, BDNF, and CHAT (mediating choline acetyltransferase) were downregulated.⁶

In summary, in established AD, studies of the entorhinal cortex and hippocampus show that either the majority of genes have downregulated gene expressions or there is an approximately equal number of downregulated as upregulated genes.

3 | IN MCI, GENE EXPRESSION IS MOSTLY UPREGULATED

The contrary pattern is seen in MCI, where upregulation of gene expression dominates. Thus, brains from 12 patients with MCI, 25 with AD, and 24 age-matched non-demented controls showed gene expression alterations in the MCI patients that were almost precisely the converse of those seen in the patients with AD.⁷ The majority of genes were upregulated in MCI relative to AD, especially those genes associated with protein function and mitochondrial energy production. In particular, gene expression for synaptic function in the hippocampus was dominantly upregulated contrasting with its overwhelming downregulation in established AD. Another study showed upregulation of mitochondrial genes expressing respiratory chain complexes I, III, IV, and V in MCI, whereas 95% of them were downregulated in AD.⁸

The very early Clinical Dementia Rating (CDR) score of 0.5 probably overlaps the clinical diagnosis of MCI. Brains from persons with CDR 0.5 had no loss of either synaptotagmin or GAP43 (a marker of neural sprouting) compared with brains from persons with CDR 0, but had significant and progressive decline in brains of persons with CDR > 19.⁹

Although some studies of MCI gave different results,¹⁰⁻¹² it is likely because the involved groups of patients had different stages of MCI. That likelihood was demonstrated by examining cognitive decline versus gene expressions in the prefrontal cortices of 49 individuals; the total group comprised seven persons within each of the seven Braak stages 0-6 (the neuropathologic stages 0-1 presumably overlap the clinical diagnosis MCI).¹³ It showed that upregulation of genes occurs in the very earliest of Braak stages (0-1), and downregulation appeared in the later stages. That study examined 29,813 gene expressions, and for each Braak stage the gene expressions were averaged and categorized as being either upregulated or downregulated. To follow the expression of individual transcripts during the course of AD, expression profiles over the consecutive Braak stages were constructed. There were two patterns of change in gene expression: in one pattern (up-then-down), 865 genes were upregulated in early Braak stages and became downregulated in later Braak stages; in the other pattern (down-then-up), 983 genes were downregulated in early Braak stages and became upregulated in later Braak stages. The most profound changes of this sort were between Braak stages 2 and 3, just before or at the onset of neurofibrillary pathology in the prefrontal cortex; in Braak stages 0-1 the majority of genes were upregulated whereas in the higher Braak stages the majority were downregulated, with the turning point for the switch occurring between Braak stages 2 and 3. The up-then-down pattern included 532 genes involved with synaptic activity and plasticity, and 1056 involved with mitochondrial function. Additional genes showing the up-then-down pattern were those related to G protein-coupled signaling, electron transport, cell-cell signaling, microtubules, calcium ion binding, and cytoplasm. A different report examined gene expression abnormalities of the key enzymes that control sphingolipid metabolism, and showed that a switch from down- to upregulation of *PPAB2B* (expressing a phosphatidic acid phosphatase causing hydrolysis of sphingosine-1 phosphate to sphingosine

and of ceramide-1 phosphate to ceramide) occurred between Braak stages 1 and 3; and similar but non-significant switches were also seen for *SPTLC2* and *LASS-1*.¹⁴

It is not only the direction of change but also the degree of gene expression that may suddenly switch. This was also seen in Braak stage 2: erythropoietin expression in astrocytes of the temporal cortex was unchanged from control levels in Braak stage 1 but increased in Braak stage 2 and remained high in stages 3–6.¹⁵ A switch in the level of expression was also seen at much more advanced Braak stages for several microRNAs (455-3p, 3613-3p, 4674, 6722).¹⁶ Figure 6 in that report shows dramatic increases in fold levels of those miRNAs in the transition from Braak stage 4 to stage 5.

In summary: In MCI there is an overall upregulation of gene expression that then switches to an overall downregulation when MCI progresses to AD; that switch mostly occurs in the transition to Braak stages 2 or 3. A switch in degree of gene expression may also occur.

4 | COLLATERAL, SUPPORTING DATA INVOLVING UNEXPLAINED, OPPOSITE MANIFESTATIONS IN MCI AND AD OF GENETICALLY CONTROLLED CONDITIONS

Epigenetic changes in gene expression are those that occur without alteration of the DNA sequence, such as by DNA methylation of cytosine residues, which suppresses gene activity. Cytosine may be methylated as 5-methylcytosine (5mC) and 5-hydroxymethyl-cytosine (5hmC). Ellison et al. found that global levels of cytosine in the brain were significantly decreased in MCI but 5hmC levels were significantly increased, suggesting heightened conversion of cytosine to 5-hydroxymethyl-cytosine; but in AD the levels of both cytosine and 5-hydroxymethyl-cytosine were similar to controls.¹⁷ Those data suggest that in MCI and AD there are different degrees of the gene expression that controls formation of 5hmC. Another study providing supporting data involved the cystine/glutamate antiporter system Xc⁻ that is encoded by the *xCT* gene, and that imports cystine into cells with a concurrent, extracellular, 1:1 counter-transport of glutamate.¹⁸ Its importance is that cystine participates in the formation of glutathione and therefore a lower level of cystine and a higher one of glutamate, imply a lower glutathione level, less antioxidant capacity, and less neuroprotection from oxidative stress. Bell et al. studied glutamatergic presynaptic boutons in the midfrontal gyrus and saw a significantly larger number per 1000 μm^2 area in MCI but a significantly smaller number in AD.¹⁹ Those data suggest that in MCI and AD there is an opposite degree of genetic control of the cystine/glutamate antiporter system Xc.

5 | POSSIBLE MECHANISMS DETERMINING THE DISPARATE RESULTS BETWEEN MCI AND AD IN GENE EXPRESSIONS

What might account for the switch from upregulation to downregulation at a time-controlled point in the progression from MCI to AD?

The answer may lie in the control mechanisms for gene expression. Those mechanisms are complex and include modification of DNA and histones by methylation or acetylation, affecting the balance between the Compass group of proteins that enhances mRNA formation, and the Polycomb group that suppresses it (for reviews, see El Brolosy and Stainier²⁰ and Piunti and Shilatifard²¹); mutations in regulatory sequences of DNA; and in the transcription factors, cofactors, chromatin regulators, and noncoding RNAs (including microRNAs), that all interact with the regulatory sequences of DNA (see Lee and Young²² for a detailed review). Although this degree of complexity renders tenuous even a tentative guess as to the determinant(s) of the switch under consideration, nevertheless there are two strong reasons to consider a potentially, participatory role for the repressor element-1 silencing transcription factor (REST), also known as the neuron-restrictive silencer factor (NRSF). First, because REST levels are substantially different between MCI and AD: as calculated from figure 1e in Lu et al.,²³ REST levels were reduced by only 40% in MCI but 1.5-fold more at 60% in AD. REST silences transcription of neuronal genes via recruiting histone deacetylases (HDACs) and histone methyltransferases²⁴; so, if appropriately timed, a lowered expression of REST could determine the switch from up- to downregulation in the progression of MCI to AD and in that way, either acting alone or with other factors, be a major determinant of progression.²⁵ For that to happen, REST must be able to act in a time-controlled fashion, and two reports show that it has that capability: (1) during rats' development it promotes the switch from GluN2B to GluN2A in the composition of the NMDAR ion channels²⁵; (2) REST determines the timing of the chloride shift from high to low intraneural chloride concentration that is caused by upregulation of *Kcc2b*, the co-transporter of neuronal KCl that controls the time-dependent GABA switch.²⁶ The seminal report by Lu et al.²³ provides details about REST repressing cell death genes and about promoting expression of the anti-apoptotic gene BCL-2, the antioxidants catalase and SOD1, and the transcription factor FOXO1a (implicated in oxidative stress resistance and longevity). In AD brains, Lu et al. saw almost total absence of REST in neuronal nuclei of the PFC and CA1, CA2, & CA3, and nuclear REST levels were significantly correlated with measures of episodic, semantic, and working memory. Lu et al. also noted that REST levels in brains from subjects of advanced age were significantly higher than in brains from young subjects. Thus, the low levels of REST in AD are disadvantageous. The extensive literature about REST shows that its effects are substantially different in pluripotent, embryonic stem cells; multipotent neural stem cells; and mature neurons. Because MCI and AD involve mature neurons, it is to those that the following refers. Collectively, several reports indicate that low levels of REST cause reduction of the neurogenic capacity of adult, neural stem cells and, thus, decreased neurogenesis. Otto et al. analyzed a library of 29,807 genomic signature tags and found that REST binding sites involved synaptic signaling, ligand receptors, transcription molecules, ion channels/transporters, and adhesion molecules.²⁷ REST was particularly linked to gene products dedicated to both presynaptic and postsynaptic neurotransmission, that is, synaptotagmins, synaptophysin, and RIM, that mediate presynaptic vesicle release, and genes encoding postsynaptic

receptors and voltage -dependent potassium and calcium channels. Using gene expression analysis, Kim et al. found that knock-down of REST in neural progenitors led to loss of function of the secretogranin2 gene (*Scg2*) plus defects in dendritic spines, which would impair neural plasticity.²⁸ Ballas et al. saw that loss of REST was associated with reduced expression of the *BDNF* and *Calbindin* genes²⁹ recalling the finding already mentioned that BDNF is downregulated in AD.² In brief, evidence suggests that the increased reduction of REST in AD of MCI, produces suboptimal levels in AD of critical gene expressions, particularly those involved in neural plasticity.

It is certainly possible that none of the above is the correct response to the question posed. "what accounts for the switch at the time of progression?" It could be simply that the potential progressors already possessed gene expressions which were the same as those in AD and that the non-progressors are those whose gene expressions remained in a direction opposite to that in established AD. Unfortunately, there are no data that either support or refute this possibility.

6 | IF REST IS THE DRIVER, ITS LEVEL MIGHT BE CONTROLLED BY A miRNA (PERHAPS miRNA-124)

As always, one question leads to another which, in the present case asks, "what determines the ~50% decreased REST level in AD compared with MCI?" Immediately one is led to consider miRNAs, which primarily are regulators of mRNA translation.³⁰ Relevant to the timing of the switch from MCI to AD, which occurs at approximately the same time as do both a changed direction of gene expression as well as the decrease of REST, there is evidence that some miRNAs affect the timing of mRNA transcription. They do so by means of synchronously inhibiting a group of functionally interdependent genes.³⁰ MicroRNAs, particularly miRNA-124, which is abundant in the brain,³¹ bidirectionally control REST levels. It is also relevant that miRNAs may act both as repressors and activators of genes depending upon the cellular circumstances.³² MiRNA-124, whose mature sequences are conserved from *Caenorhabditis elegans* to humans, is the most abundant miRNA in the adult CNS.³³ MiRNA-124 is expressed by neuroblasts in the adult SVZ niche and knockdown of miRNA-124 by an antisense RNA caused an increase of both dividing neuroblasts and transit amplifying cells.³⁴

7 | AN EXPERIMENT COULD DEMONSTRATE IF REST ACCOUNTS FOR THE SWITCH FROM MCI TO AD

The foregoing suggests an informed guess that places miRNA-124 and REST as central to the progression from MCI to AD. MiRNA-124 is commercially available. Experiments using passive or active immunity (either would be easy to induce in rodents), or a specific antisense oligonucleotide, in rodents transgenic for AD, could demonstrate whether inhibiting miRNA-124, and thereby increasing REST levels,

prevents progression to AD in that model. Results of such experiments, if they showed that inhibiting miRNA-124 impaired progression from to AD, could become clinically useful.

8 | CONCLUSIONS

During the progression of MCI to AD, there is a shift of the direction in which genes are expressed, from being dominantly upregulated in MCI to being dominantly downregulated in AD. This switch occurs mostly when Braak stage 0–1 progresses to stage 2–3. Candidates for the explanation of the mechanism underlying the switch include REST. Because miRNA-124 inhibits REST in a time-controlled fashion, experimental inhibition of miRNA-124 in a rodent, AD model, might demonstrate whether this approach has potential clinical merit.

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