

# Anticipated Brain Molecular Aging in Major Depression

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**Objectives:** Brain molecular aging, the pervasive and consistent transcriptome changes associated with normal brain aging, appears to overlap with disease pathways and may be anticipated in neurodegenerative and neuropsychiatric diseases, including major depressive disorder (MDD). Here, we characterize the global interaction of MDD-related gene changes with age, starting from our previous report of downregulated brain-derived neurotrophic factor (BDNF) and BDNF-dependent genes in the amygdala of women with MDD. **Methods:** A large-scale gene expression data set in the amygdala from a postmortem cohort of 21 women with MDD and 21 age-matched controls (age range: 16–74 years) was analyzed for correlations of gene transcript changes with age, in the presence or absence of a diagnosis of MDD. **Results:** 1) The age-related decrease in BDNF transcripts observed in control subjects corresponds with further age-related decreases in BDNF and BDNF-dependent gene expression in MDD subjects; 2) most MDD-related genes are frequently age-regulated in both MDD and control subjects; 3) the effects of MDD and age are positively correlated; 4) most genes that are age-dependent in control subjects display greater age effects in MDD subjects; and 5) the increased prevalence of age effects in MDD corresponds to similar trends in controls, rather than representing *de novo* age effects. **Conclusions:** MDD strongly associates with robust and anticipated gene expression changes that occur during normal aging of the brain, suggesting that an older molecular age of the brain represents an early biological event and/or a marker of risk for subsequent onset of MDD symptoms. (Am J Geriatr Psychiatry 2013; 21:450–460)

**Key Words:** Aging, amygdala, BDNF, brain, human, major depression, microarray, neuropsychiatric, post mortem

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Along with increased lifespan comes an increased risk for neuropsychiatric and neurological disorders.<sup>1</sup> Studies of the functional correlates of aging consistently report increased incidence of low

mood, motor deficits, and declines in certain cognitive functions.<sup>2–4</sup> Mood disorders per se do not necessarily increase with age, but studies report increasing numbers of depressive symptoms in older

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individuals; hence, the underdiagnosing of depression may potentially reflect diagnostic structure and procedural issues, because older individuals tend to underreport psychiatric symptoms and psychiatric symptoms can be perceived as part of normal aging (see Fiske et al.<sup>4</sup> and McKinney et al.<sup>5</sup>). Low mood also occurs in presymptomatic phases of neurodegenerative disorders. Together, these findings suggest that aspects of mood regulatory mechanisms may be selectively vulnerable during normal and pathological aging or that depressive symptoms may represent a common output for various underlying age-related brain declines.<sup>4,6</sup> Although it is difficult to untangle causal links, evidence suggests that mood symptoms may represent an early marker for subsequent spiraling functional declines.<sup>3</sup>

On the contrary, depression has been compared with a state of “accelerated aging,” because subjects with depression display an increased incidence of various diseases of aging, such as cardiovascular and cerebrovascular diseases, metabolic syndrome, and dementia.<sup>7,8</sup> However, the mechanisms underlying normal aging processes and disorders of old age are still being characterized.<sup>9,10</sup> Specifically, recent progress in investigating the molecular, cellular, and neural bases of depression in adult subjects, and the observation of a continuum of changes between normal aging and depression-related changes, have suggested a framework where molecular aging of the brain may promote biological changes that may in turn place the system at higher vulnerability for several age-related diseases.<sup>5,9,10</sup> Specifically, age-related gene expression changes have been described in the human brain and affect approximately 10% of all genes.<sup>11–18</sup> These changes in gene transcript levels are continuous throughout adult life, include both upregulated and downregulated genes, affect specific cellular functions, and overlap both in terms of affected genes and direction of change with those observed in the context of neuropsychiatric disorders.<sup>11,18</sup> For instance, gene transcript levels for brain-derived neurotrophic factor (BDNF) decrease with age in the human brain,<sup>11,18,19</sup> but BDNF levels have also been reported to be low in the context of neuropsychiatric disorders.<sup>20–23</sup> Note that these are cross-sectional measures; hence, because the actual longitudinal molecular trajectories are not known, we use here the term “anticipated” rather than “accelerated” aging.

We recently reported robust large-scale gene expression changes in the amygdala in the postmortem brains of women affected with major depressive disorder (MDD) compared with control subjects (see details and discussion in Guilloux et al.<sup>22</sup>). MDD-related changes included a significant downregulation of BDNF, which also correlated with similar changes in a set of BDNF-related genes. Using analyses of covariance, age was identified as a significant covariate for BDNF and BDNF-related gene signals, while multiple additional clinical and demographic parameters did not affect the findings.<sup>22</sup> Here, in the broader context of an age-by-disease interaction hypothesis,<sup>5,10</sup> we investigated these prior findings in more detail in the same cohort. Specifically, we hypothesized that MDD-related gene changes, in addition to those related to BDNF, 1) would correlate with chronological age across subjects; 2) would correspond to normal age effects in control subjects; and 3) that the magnitude of age-related changes would be greater in MDD subjects.

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## METHODS

### Human Postmortem Subjects

After consent was obtained from the next of kin, brain specimens were obtained during autopsies conducted at the Allegheny County Medical Examiner's Office (Pittsburgh, PA). For all subjects, consensus *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* diagnoses of MDD were made by an independent committee of experienced clinical research scientists at a case conference, utilizing information obtained from clinical records, toxicology examination, and a standardized psychological autopsy.<sup>24</sup> The standardized psychological autopsy incorporates a structured interview, conducted by a licensed clinical psychologist with family members of the index subject, to assess diagnosis, psychopathology, medical, social, and family histories, as well as history of substance abuse. Rates of death by suicide, disease recurrence, evidence for antidepressant treatment at time of death, and alcohol dependence in MDD subjects were recorded. Toxicologic screens on peripheral fluids were used to identify the presence of antidepressant medication. Twenty-one pairs of subjects were analyzed, each pair consisting of one female subject with MDD and one control subject

matched for sex, race (88% white), and as closely as possible for age, postmortem interval (PMI) and brain pH (for complete details of the cohort, see Table S1, Supplemental Digital Content 1; available online and Guilloux et al.<sup>22</sup>). Brains were analyzed for adequate brain pH (>6.4) and ribonucleic acid (RNA) integrity by optical density ( $OD \geq 1.6$ ) and Agilent bioanalyzer analysis (Agilent Technologies, Palo Alto, CA; RNA integrity number [RIN] expert scoring system  $\geq 7$ ) as described.<sup>25</sup> Accordingly, subject groups did not differ in mean age, PMI, brain pH, RIN, or RNA ratio ( $p > 0.05$ ).

### Microarray Samples

The sample collection and generation of gene microarray data was described in Guilloux et al.<sup>22</sup> In brief, rostral amygdala samples enriched in lateral, basolateral, and basomedian nuclei were dissected from frozen coronal blocks approximately 2 cm caudal to the temporal pole. Total RNA was isolated and assessed using the Bioanalyzer 2100 (Agilent Technologies, Walbronn, Germany). To reduce the influence of technical variability, paired samples were processed together, but different pairs were randomly distributed at each experimental step. Note that 1  $\mu\text{g}$  of total RNA was reverse-transcribed and converted into double-stranded complementary deoxyribonucleic acid. A biotinylated cRNA was transcribed in vitro, using an RNA polymerase T7 promoter site introduced during the reverse-transcription step. Fragmented labeled cRNA sample was hybridized onto Human Whole-Genome Expression Beadchips (Human HT12 v4.0, Illumina, Inc., San Diego, CA), assessing around 31,000 annotated genes. Probeset signals (i.e., transcript levels) were extracted and batch-normalized with the Beadarray (Illumina, Inc., San Diego, CA) software following the default quality control parameters.

### Real-Time Quantitative PCR

Quantitative polymerase chain reaction (PCR) was performed as previously described.<sup>25</sup> Small PCR products were amplified in quadruplets on a Mastercycler real-time PCR machine (Eppendorf, Hamburg, Germany). Results were averaged across the four replicates and transformed into relative expression levels ( $2^{-\Delta\Delta C_t}$ ) as the geometric mean of relative intensities compared with three internal controls (actin, glyceraldehyde-3-phosphate dehydrogenase,

and cyclophilin) with comparable expression in both subject groups.

### Statistical Analyses

*Statistical Analysis of Disease Effect.* The selection of MDD-related genes was made using a random-intercept statistical model (RIM) to adjust for clinical, demographic, and technical variables (antidepressant treatment, suicide, severity, pH, PMI, and RIN), as described in Guilloux et al.<sup>22</sup> and reproduced later. Alternatively, parametric (paired *t*-test) or nonparametric (Wilcoxon test) statistical tests were also applied. Their respective use is described in the "Generation of Gene Lists" section later.

RIM was applied to account for paired design (MDD samples paired with corresponding controls with no disease) and existence of clinical, demographic, and technical variables. For a given gene  $g$ , we fit the model  $Y_{gik} = \mu_g + \beta_{g0}X_{0ik} + \sum_{i=1}^L \beta_{gi}X_{lik} + \alpha_k + \epsilon_{gik}$ , where  $Y_{gik}$  is the gene expression value of gene  $g$  ( $1 \leq g \leq G$ ) and sample  $i$  ( $i = 1$  for controls and  $i = 2$  for MDD) in pair  $k$  ( $1 \leq k \leq K$ ),  $X_{0ik}$  takes one if the sample is MDD and zero if the sample is a control subject,  $X_{lik}$  represents values for clinical variables (e.g., 0–1 binary for respective parameters, i.e., taking alcohol or not, antidepressant exposure, or numerical for brain pH or PMI values) and  $\alpha_k$  is the random intercept, which are the deviations of average of all expression values at  $k$ th pair from the average in the whole population and is assumed to follow normal distribution with mean zero and variance  $t_g^2$ . Finally,  $\epsilon_{gik}$  are independent random noises with normal distribution with mean zero and variance  $s_g^2$ . Under this model,  $\beta_{g0}$  is the disease effect of gene  $g$  of interest to be estimated. To obtain a MDD-associated biomarker list, likelihood ratio test (LRT) is used to assess the  $p$  values of testing  $H_0: \beta_{g0} = 0$  and  $p$  values are then corrected by the Benjamini–Hochberg procedure for multiple comparison,<sup>26</sup> with a false discovery rate (FDR) set at 5%.

*Generation of Gene Lists.* List 1 (exploratory) was obtained using uncorrected parametric and nonparametric statistical tests (RIM, paired *t*-test, and Wilcoxon test), with no threshold on effect size for MDD effect, resulting in an exploratory list of 4,131 genes with uncorrected statistical differences between MDD and control subjects.

List 2 (intermediate) contained 307 genes obtained using uncorrected parametric and nonparametric

statistical tests (RIM, paired *t*-test, and Wilcoxon test), moderate statistical stringencies for gene selection ( $p < 0.05$  in at least one statistical test), and MDD effect size greater than 20%.

List 3 (stringent and FDR-corrected) was composed of gene selection made using RIM to adjust for clinical, demographic, and technical variables, followed by correction for multiple testing by the Benjamini–Hochberg method,<sup>26</sup> resulting in a stringent selection of 116 differentially expressed genes (adjusted *p* value, i.e., *q* value:  $< 0.05$ ; mean change:  $> 20\%$ ).

*Statistical Analysis of Age Effects on Gene Transcripts.* For each individual gene, we calculated the individual ratio of gene expression to the average expression of this single gene. For age effect, a Pearson correlation was performed using individual gene expression values and age, in the control population and the MDD population. Based on a cohort of 21 subjects (control or MDD), a Pearson correlation factor  $> 0.369$  or  $< -0.369$  gives  $p < 0.05$ . Changes in slopes of combined age-dependent gene changes were calculated by Student's *t*-tests, using slope values for the same individual genes between the control and MDD subjects. The relative proportions of age-related genes were compared between groups using the  $\chi^2$  test.

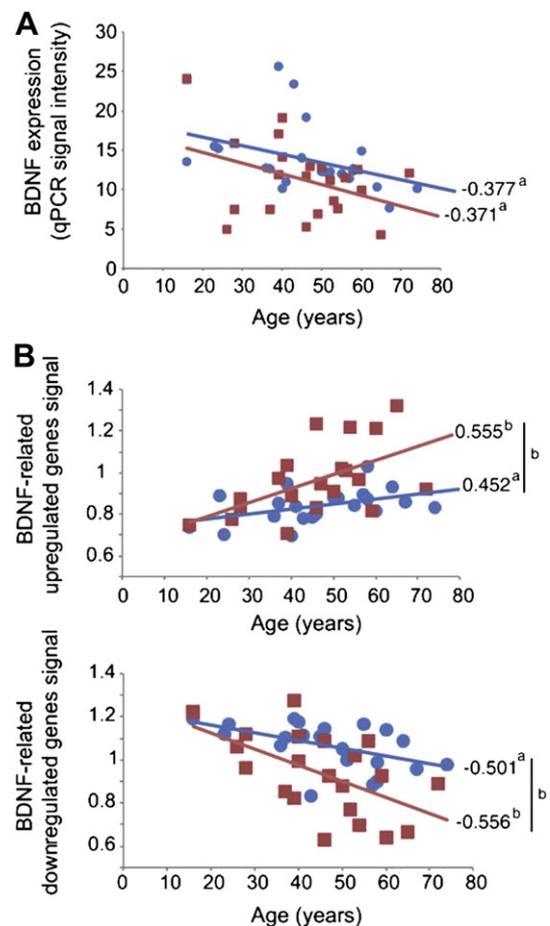
## RESULTS

### Reduced BDNF Expression With Age and MDD Associates With Greater Age Effects on BDNF-Related Genes in MDD Subjects

We previously reported that disease-associated genes, including BDNF, are robustly modulated by age in control subjects<sup>11,18</sup> and we also described a correlation with age for several genes that are under the control of BDNF in a cohort of postmortem female subjects with MDD (see Supplementary Figure S3 in Guilloux et al.<sup>22</sup>). Here, we further investigated the relationships between age, MDD, and altered transcript levels, starting with changes in BDNF-related genes in subjects with MDD compared with control subjects.

Displaying quantitative PCR measures as a function of age, we confirm that BDNF mRNA expression decreases with age, in both the control and MDD groups, with a greater mean reduction in MDD subjects (Figure 1[A];  $-22\%$ ,  $p < 0.05$ , with respect to

**FIGURE 1.** BDNF and BDNF-related gene expression decreases with age. [A] BDNF expression, measured by quantitative PCR, is significantly and inversely correlated with chronological age in control and MDD subjects. Values are in arbitrary units of quantitative PCR, signal intensity. Respective to age-matched control subjects, MDD subjects display greater BDNF downregulation ( $-22\%$ ;  $p < 0.05$ ; Guilloux et al.<sup>22</sup>). [B] Age regulation of BDNF- and MDD-related genes. The average relative age effects for each individual subject are shown for the set of BDNF-related genes that display significant MDD effects. Microarray-based gene expression values were normalized to the group means of each gene and averaged per subject. The BDNF-related gene set was split based on the effect of age on those genes in control subjects (top panel, age upregulated; bottom panel, age downregulated). The results show that age effects are systematically in the same direction and of greater effect sizes in MDD subjects (red squares) than in controls (blue circles). Values are Pearson correlation factors.



<sup>a</sup> $p < 0.05$ ; <sup>b</sup> $p < 0.01$ .

age-matched control subjects), but no difference in slope values ( $p = 0.133$ ). Because BDNF is a neurotrophic factor that regulates the expression of many downstream genes, we tested whether MDD related genes that are also BDNF-related may display greater change with age than do control subjects. From an exploratory gene list for MDD effect in the amygdala-based microarray data set in a cohort of 21 female MDD compared with 21 matched controls (see list 1 in the “Methods” section), 52 genes were identified as BDNF-related through the presence of literature-based links in the Ingenuity database. BDNF-dependent genes were then split based on the direction of MDD effect on gene expression (33 upregulated and 19 downregulated; see Table S2, Supplemental Digital Content 2; available online). Individually, 11 of these 52 BDNF-related genes displayed significant age effects in controls (4 upregulated and 7 downregulated genes with age), compared with 26 in the MDD cohort (8 upregulated and 18 downregulated genes with age). The combined age effects for these MDD upregulated or downregulated genes within each subject are displayed in Figure 1[B] as a function of age. As expected from our prior combined studies,<sup>22</sup> these average values displayed significant positive correlation with age for MDD-upregulated genes and significant negative correlation with age for MDD-downregulated genes. Notably, although age-dependent changes display parallel trajectories for BDNF (Figure 1[A]), cross-subjects trajectories of combined changes for BDNF-dependent genes displayed increasing effect sizes with age in MDD subjects (Figure 1[B]; MDD versus control slope differences: upregulated genes,  $p = 0.006$ ; downregulated genes,  $p < 0.0001$ ).

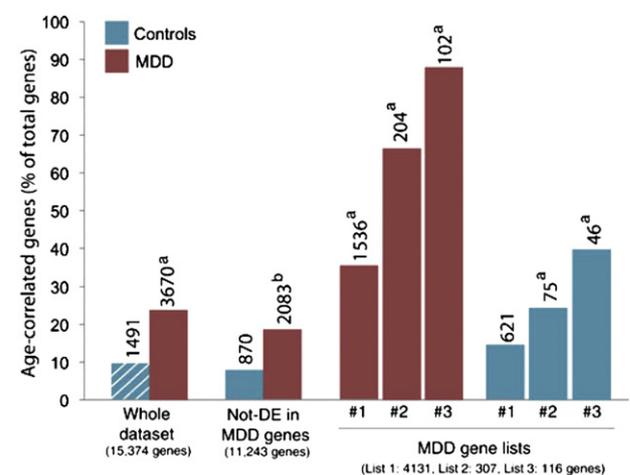
These results demonstrate that gene expression changes for BDNF in MDD display similar age correlation, although to a higher extent and age-dependent rate than control subjects, together suggesting that normal age-related changes may contribute to MDD-related biological changes.

### Greater Numbers of Genes Affected by Age in MDD Subjects and Overrepresentation of Age Effects on MDD-Related Genes in Control Subjects

To test whether the increased age effect on MDD-related genes in MDD subjects was limited to BDNF-related genes, we then assessed the relative

frequencies of genes displaying significant correlations between transcript levels and chronological age (i.e., age effect) for several sets of genes, based on increasing statistical stringency for MDD effects. The baseline for age effect was assessed in control subjects, where 9.7% of the genes ( $n = 1491$ ) displayed significant age effects (Pearson correlation with age,  $p < 0.05$ ; see the “Methods” section; Figure 2, hashed bar column), consistent with prior findings.<sup>18</sup> In comparison, 3,670 genes were correlated with age in MDD subjects (23.9%;  $\chi^2 = 22.9$ ,  $p < 0.0001$ ; Figure 2). Restricting the pool of genes to only those affected in MDD progressively increased the relative proportion of genes significantly correlated with age (Figure 2; list 1: 35.3%,  $\chi^2 = 74.9$ ,  $p < 0.0001$ ; list 2: 66.4%,  $\chi^2 = 367.7$ ,  $p < 0.0001$ ), reaching a remarkable 87.9% in the list with the most stringent criteria for MDD effects (Figure 2; list 3:  $\chi^2 = 698.7$ ,  $p < 0.0001$ ). Notably,

**FIGURE 2. Increased numbers of age-correlated genes in MDD subjects. The percentages of age-related genes (y-axis) in MDD (red bars) and control subjects (blue bars) are displayed for various lists of genes with increasing stringency for MDD effects (list 3 is the most stringent; see the “Methods” section). The numbers on top of bars represent the number of age-related genes. The numbers in parentheses at the bottom of the columns represent the number of genes in the corresponding gene lists. <sup>a</sup> $p < 0.0001$  and <sup>b</sup> $p < 0.01$ , using  $\chi^2$  analyses, comparing the frequency of age-dependent genes in the different groups to the expected frequency, that is, the frequency of age-correlated genes observed in the control group (blue bar with white dashes). DE: differentially expressed.**



a similar trend of increased relative proportion of age effects was observed for the same gene lists in the control subjects (Figure 2; list 1: 14.3%,  $\chi^2 = 2.42$ ,  $p = 0.11$ ; list 2: 24.4%,  $\chi^2 = 24.7$ ,  $p < 0.0001$ ; list 3: 39.6%,  $\chi^2 = 102.5$ ,  $p < 0.0001$ ).

Increased age effects in MDD subjects were also observed for genes that did not display MDD-related changes compared with the expected frequency, that is, the frequency of age-correlated genes observed in the control group (Figure 2, blue bar with white dashes), as demonstrated by a relative increase in age effect in that gene list (Figure 2; "Not-differentially-expressed [DE] in MDD genes"; red bar, 18.5%,  $\chi^2 = 10.7$ ,  $p = 0.0011$ ). In comparison, the same gene list (not-DE in MDD) did not show an increased proportion of age effect in control subjects (Figure 2: "not-DE in MDD"; blue bar, 7.7%,  $\chi^2 = 0.44$ ,  $p = 0.5$ ).

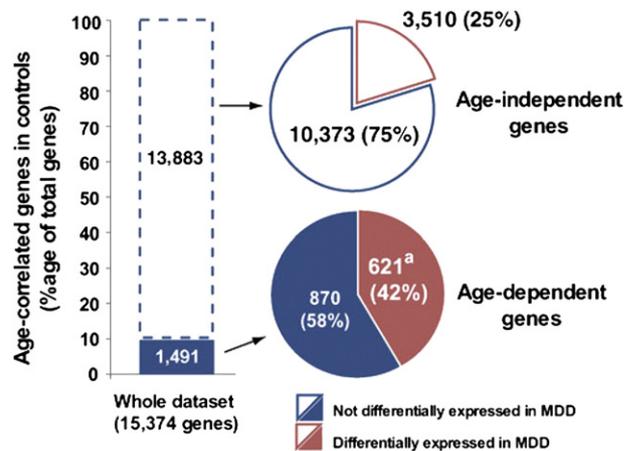
Together, these results demonstrate that the overlap between the MDD-related pathology and the gene correlates of aging extends well beyond the indirect effects of decreased BDNF on gene transcript levels, and in fact suggests that the presence of MDD accentuates the propensity of a gene to be age-regulated.

**Age Correlation in Control Subjects Predicts Greater Risk of Association With MDD**

Conversely, the increased probability for any gene that is affected in MDD to also display age-related changes in control subjects, combined with the absence of increased age effects in the list of genes not affected in MDD (Figure 2, blue bar compared with blue bar with white dashes), suggests that age dependency in control subjects may predict putative involvement in MDD pathology. Accordingly, Figure 3 shows that 42% of the genes significantly correlated with age in control subjects were also differentially expressed in MDD subjects (Figure 3, lower pie chart). This proportion is significantly greater than the 26.9% of genes displaying significant MDD correlation for the whole data set ( $\chi^2 = 11.11$ ,  $p < 0.001$ ). On the contrary, age-independent genes showed a nonsignificant difference in the rate of association with MDD compared with the whole data set (Figure 3; 25.3%;  $\chi^2 = 0.13$ ,  $p = 0.72$ ; upper pie chart).

Together, these results demonstrate that the set of genes displaying age-dependent changes in control subjects largely overlaps with the set of genes that is

**FIGURE 3. Overrepresentation of MDD effects in gene set displaying age-dependent changes in controls.** The histogram shows the relative proportion of genes showing age effects in the amygdala of control subjects (age-dependent, dark blue, approximately 10%). The lower pie chart indicates that 42% of the age-dependent genes in controls are also differentially expressed in MDD subjects (based on list 1, see the "Methods" section). \* $p < 0.0001$ ;  $\chi^2$  analysis comparing frequency of genes differentially expressed in MDD in the age-dependent genes selection compared with their frequency in the whole data set. The upper pie chart indicates that the frequency of MDD effect in the set of genes that are age independent was nominally greater, but not significantly different, from the control population.



affected in MDD, and suggest that genes which are age-dependent in control subjects may be more vulnerable to the pathophysiologic processes of MDD.

**The Increased Age Effect on MDD-Related Gene Changes Corresponds to an Increase in Effect Size of Subthreshold Age-Related Changes in Control Subjects, Rather Than a True De Novo Age Effect**

Our results show that genes differentially expressed in MDD are for the most part affected by age in control subjects. However, the lists of MDD-related genes also contain genes that do not display significant age effects. Therefore, we next investigated whether this increased in age-dependency for MDD-related genes represented an increase in subthreshold age effect in control subjects (i.e., trend-like) or a true de novo agedependency effect (i.e., unique to MDD subjects).

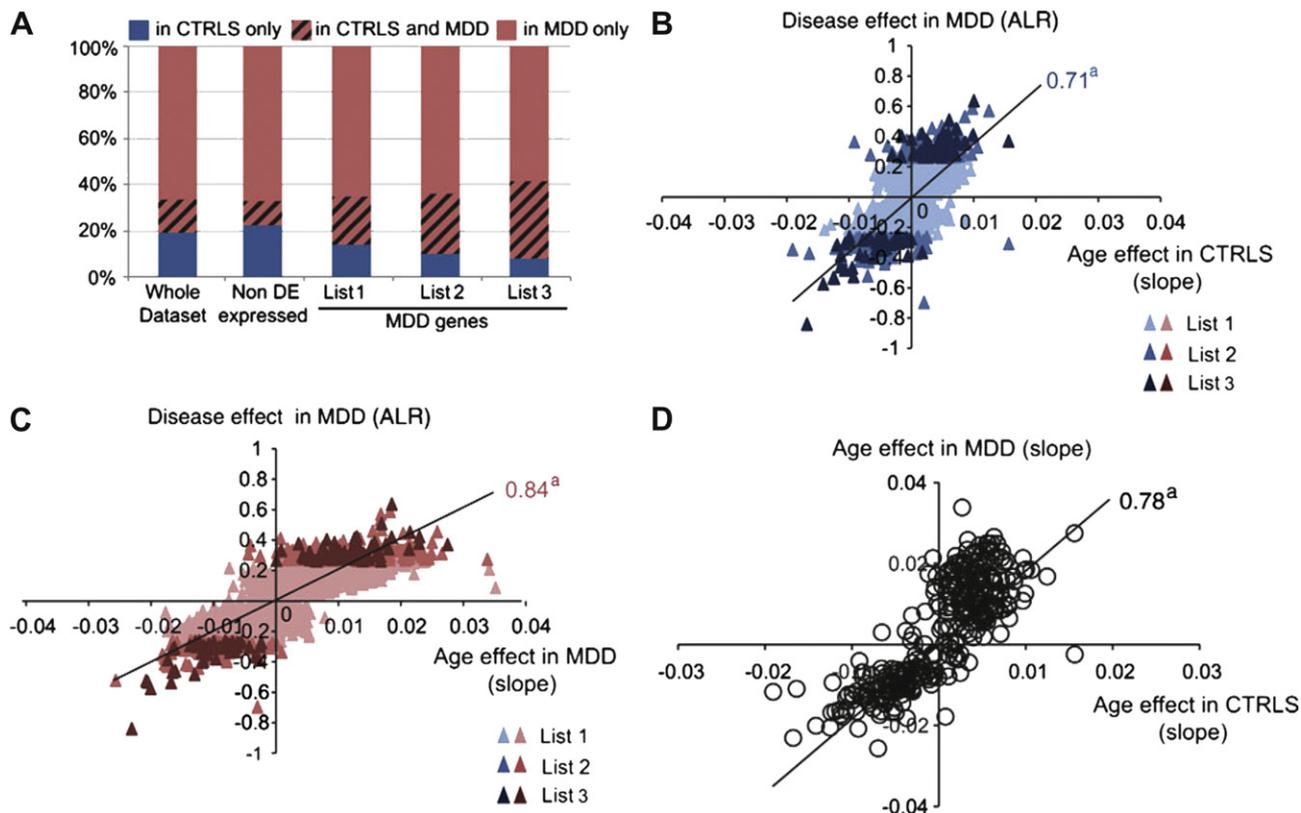
## Anticipated Brain Molecular Aging in Major Depression

We first addressed this question by showing that the relative proportion of MDD-related genes that display significant age effects solely in control subjects greatly decreases as the stringency for MDD effect increases (Figure 4[A], from lists 1 to 3). This demonstrates that any core MDD gene that is affected by age in controls will almost systematically be affected by age in the context of MDD.

We next investigated correlations between significant MDD effects and age-related changes in gene

expression in control subjects, regardless of age significance in the control group. For this, we speculated that in control subjects, the slope values of the cross-subjects trajectory of changes in expression with chronological age may capture small trends in gene changes that would otherwise not reach statistical significance for age correlation. Accordingly, we show that the extent of MDD effect on gene transcript levels (average log ratio of MDD versus control signal) is positively correlated with the slope values of age

**FIGURE 4.** MDD-related gene changes are globally and positively correlated with age in MDD subjects, and are positively correlated with subthreshold effects in control subjects. [A] The relative proportion of genes correlated with age solely in controls (blue) decreases in gene lists with increasing stringency for statistical association with MDD. Red indicates proportion of genes with age effects solely in MDD subjects. The red blocks with black hashed lines indicate the proportion of genes displaying age effects in both subject groups. [B and C] Correlation between the effect of MDD (average log ratio [ALR] value of the MDD effect) and the age-related effect on gene expression ratio in controls (CTRLS) [B] and MDD subjects [C]. Age effects were determined here using the slope of the linear regression of gene expression ratio as a function of age. Note that the unit of slope values (x-axis) corresponds to changes per year in expression values that are normalized to the mean expression of the respective genes, as in Figure 1. Therefore, a value of 1 corresponds to the mean expression value and slope values ranging from 0.01 (in controls) to 0.03 or 0.04 (in MDD) correspond to approximately 1% or 3%–4% of change per chronological year. [D] Correlation of effect of age in controls with effect of age in MDD population. The values in graphs [B]–[D] are Pearson correlation factors.



<sup>a</sup>p < 0.0001.

related trajectories in control subjects (Figure 4[B]). We also show that the spread of the age-related slope values increases in MDD subjects (Figure 4[C]; see x-axis spread compared with Figure 4[B]). Finally, Figure 4[D] directly compares the age-related slope values for MDD-related genes between MDD and control subjects. The tight correlation ( $R = 0.77$ ,  $p < 0.0001$ ) and elevated slope ( $>1.8$ ) of this latter comparison demonstrate that the age-related changes of MDD-related genes correspond to an almost doubling of already-existing age-related trends in control subjects, consistent with the differences in slope values observed in Figure 1 for the restricted set of BDNF-related genes (Figure 1[B]). This effect is illustrated here for genes corresponding to MDD list 2, but is observed for all selections (see Figure S1, Supplemental Digital Content 3; available online). Notably, the tight scatter of the data points along the trend-lines in Figures 4[B–D] indicates that these effects are not driven by outlier values.

Together, these results are consistent with the notion that the gene expression correlates of MDD are globally and positively correlated with age and that these effects represent increased effect sizes of small or sub-threshold age effect in control subjects rather than de novo changes of age-related trajectories in MDD subjects.

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## DISCUSSION

Expanding from our previous findings of a low BDNF expression in MDD and during normal aging in the amygdala of adult women affected with MDD compared with control subjects,<sup>22</sup> we now show that most MDD-related genes are age regulated both in MDD and in control subjects and that gene expression changes in MDD and aging are in the same direction. We also show that most genes that are age dependent in control subjects display greater age effects in MDD subjects and that age correlation in control subjects predicts a greater likelihood of association with MDD. Finally, we report that the overall increased prevalence of age effects on genes in MDD subjects corresponds with similar changes in control subjects, although of reduced effect size, rather than to de novo age effects. Together, these results demonstrate that MDD is strongly associated with robust and anticipated gene expression changes that occur during normal aging of

the brain, and suggest that normal aging of the brain, as measured by changes in gene transcript levels, may represent an early biological event in the disorder, which potentially accelerates with older age. Indeed, since many age-related gene changes are affected in MDD in the same direction and to a greater extent, we speculate that normal age-related events can contribute to the pathophysiologic processes engaged in MDD and that the impact of MDD on gene expression becomes greater as subjects become older.

Arguments in support of an “older brain” in MDD have been reviewed in detail by Wolkowitz et al.,<sup>7,8</sup> with an emphasis on stress-related comorbid conditions, such as vascular diseases, metabolic syndrome, and neurosteroid dysregulation, contributing to the biological profile that is observed in MDD subjects and that suggests a state of accelerated aging. The reviewed evidence suggests that disruptions in multiple cellular pathways, which occur downstream from stress and other metabolic disturbances, may lead to systemic and brain diseases, including depression, and overall accelerated cell aging.<sup>7,8</sup> The data presented here overall fit with the presence of an “accelerated” or “anticipated” state of older biological age. That is, our results demonstrate that age- and MDD-related gene changes greatly overlap and, specifically, that expression changes that occur in the context of MDD for a specific subset of genes are actually very similar in nature, but greater in effect size and rate of change, to what happens in the normal brain during aging. An alternative hypothesis is that an older brain is less able to tolerate the effects of MDD, although it is less likely as our assay measures age trajectories in subjects covering a large age span, including subjects in early adulthood. Furthermore, when considering all genes, although 42% of age-dependent genes were also affected in MDD, a large proportion of age-dependent genes in control subjects (58%) were unchanged in MDD (Figure 3), arguing against a global increase in age effect in MDD, and rather suggesting selectivity in anticipated age changes in MDD. On the contrary, our data also show that the majority of MDD-related genes changes correspond to similar age-dependent changes in controls, even if of small magnitude. It is also possible that small effects that correspond to few percentage points may remain biologically silent in control subjects and that the moderate increases in effect size observed in MDD subjects may be sufficient to impact

the function of associated biological systems. Therefore, in short, the anticipated brain molecular aging profile that is observed in MDD is robust, not global, and does not represent *de novo* age effects. This observation of MDD affecting a subset of age-dependent genes is consistent with our prior finding showing that the global “molecular age” of individual subjects, that is, the predicted chronological age of a subject based on linear regression for all age-affected genes, did not differ between MDD and control cohorts.<sup>11</sup> Our current results suggest, instead, that predictions of molecular ages based on gene changes within more restricted biological pathways may more accurately identify relevant biological changes at the intersection of age and diseases. We are currently pursuing these lines of research in this and other larger cohorts.

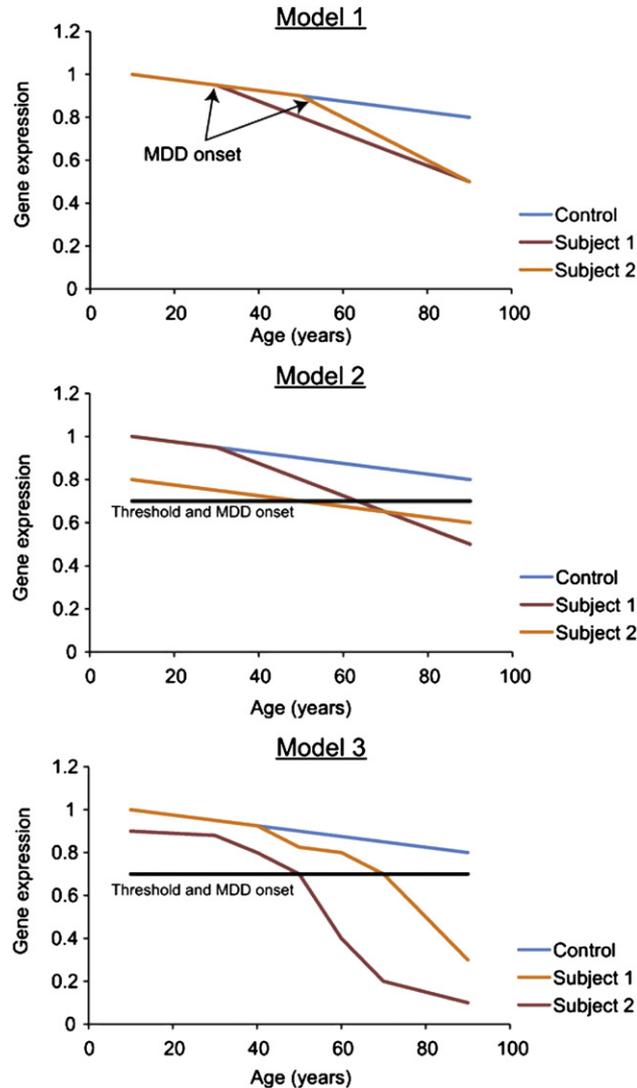
These latter considerations may also provide an answer to the basic but valid question: why are most older individuals not suffering from depression, if normal brain aging promotes biological changes that are similar to those observed in depressed patients? Therefore, selective versus global effects may be one answer (discussed earlier), but we also need to consider differences in terms of biological context and trajectory over time. First, in terms of biological context, normal molecular aging affects approximately 10% of all genes and has been hypothesized to represent the combined output of maturation processes, deleterious events (e.g., oxidative damage and neurosteroid dysregulation), and compensatory mechanisms that together contribute to maintaining homeostasis in the face of a changing biological and biochemical environment and in the context of the genetic and lifetime history of the individual.<sup>9,10,18</sup> Therefore, it is likely that molecular aging has a strong adaptive component and that disruptions of subsets of age-dependent genes may offset this homeostatic state, without necessarily affecting global molecular aging. Second, in terms of trajectory of changes, MDD correlates with numerous changes in gene expression that have, at best, very small age-related effects on gene transcripts in control subjects, which on their own may never reach threshold for disrupted functions leading to MDD (Figures 2 and 4). Finally, a progressive normal loss of approximately 40%–50% of BDNF expression in 50 years may have a very different impact on neurobiological processes, compared with a more drastic loss of 50%,

which is reported to occur in depressive episodes, as measured by circulating blood levels.<sup>27</sup> Although the origin and trajectory of BDNF loss in MDD is not known, peripheral measures suggest that BDNF changes in MDD correlate with depressive episodes and recovery,<sup>27</sup> demonstrating a plasticity of BDNF changes in MDD that is most likely very different from that of aging. Here, because the majority of subjects were depressed at the time of death (less than 25% in partial remission), we could not test the prediction of milder age-related deviations in recovered MDD subjects. Therefore, together, the emerging picture is that of a distorted age profile in MDD, with some age-related genes displaying normal trajectories and others displaying much greater changes or amplified subthreshold effects.

Thus, if anticipated molecular aging is observed in MDD subjects, the next set of unresolved questions is as follows: what is the actual trajectory of these changes and what could be the precipitating factors? Here, for heuristic purposes, and because we cannot currently follow actual brain molecular trajectories within individual, we speculate on putative trajectories to illustrate possible relationships between aging and depressed states. The first possibility is that the level of MDD-related gene expression is at control level prior to the onset of MDD (Figure 5, model 1). The onset of the pathology causes drastic changes in gene expression and accelerates molecular aging. Second (Figure 5, model 2), parallel and/or independent trajectories of altered gene expression eventually reach a threshold of disease onset, and genes may reside on “vulnerability trajectories” from variable length of times. Precipitating factors could be stress, cardiovascular accidents, early-life events, pregnancy, etc. A combination of the two models could also be hypothesized, where early changes are accelerated by precipitating factors (Figure 5, model 3). Conversely, protecting factors may place molecular changes on decelerated or “resilient” trajectories, hence forming the basis for individual variability in disease onset and presentation (not shown; see McKinney et al.<sup>5</sup>).

There are numerous limitations to this study. The first limitation is that all conclusions are drawn from cross-sectional observations, because longitudinal studies of molecular changes are not feasible in the human brain. Moreover, we cannot extrapolate on the expression level of MDD-related genes before MDD

**FIGURE 5.** Putative trajectories of gene expression changes underlying aging and onset of MDD (see the “Discussion” section for details). **Model 1:** the onset of the pathology causes drastic changes in gene expression and accelerates molecular aging. **Model 2:** independent trajectories of altered gene expression reach a threshold of disease onset after variable length of times. **Model 3:** in a combination of the first two models, early changes are accelerated by precipitating factors and reach threshold of disease onsets at variable times.



episodes or prior to the beginning of the pathology. Second, the expression levels of genes were measured using combined gray matter tissue samples, so small changes in expression levels or in the magnitude of changes over time may represent either true small changes, which may remain biologically silent in control subjects (as discussed earlier), or changes of greater magnitude in subsets of cells for which the effect size would be diluted in the combined samples. The latter point will need to be addressed by

single-cell studies, using laser capture microdissection for instance. The third limitation is that we have not tested for a causality relationship, and specifically investigated whether the overlap of age-related changes and aging actually cause MDD-related symptoms. Genetic studies in rodent systems may provide appropriate models for testing cause and effect. Finally, these results were obtained in a cohort of women with relatively severe MDD profiles (33% death by suicide; 71% recurrent MDD) and will need

to be confirmed in independent and larger cohorts, where the potential moderating effects of multiple factors, such as gender, disease severity, and other clinical variables (i.e., death by suicide, age of onset, and comorbid condition) can be fully evaluated.

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