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SHORT TAKE

Normal human aging and early-stage schizophrenia share common molecular profiles

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Summary

We examined genome-wide expression datasets from human prefrontal cortex of normal and schizophrenic individuals ranging from 19 to 81 years of age. We found that changes in gene expression that are correlated with aging in normal subjects differ dramatically from those observed with aging in schizophrenic subjects. Only 2.5% of genes were correlated with age in both groups. Surprisingly, we also found a significant overlap (29–34%) between those genes whose expression was correlated with aging in normal subjects and those significantly altered in subjects with early-stage schizophrenia (within 4 years of diagnosis). This suggests that schizophrenia onset anticipates the normal aging process, and further, that some symptoms of aging, i.e. dementia and psychosis, might be explained by these common molecular profiles.

Key words: age; aging; Alzheimer's; antipsychotic; microarray; psychiatric; real-time PCR.

Schizophrenia afflicts 1% of the general population, with onset in the late teens or early adulthood. In his original report, (Kraepelin, 1971 (original 1919)) described schizophrenia as a chronic deteriorating psychiatric disorder characterized by rapid cognitive disintegration, calling it 'dementia praecox' (premature dementia). While the degenerative nature of schizophrenia is controversial, studies have demonstrated that brain structural features, as well as predominant symptomatology change through the course of illness (Lieberman, 1999; Hulshoff Pol & Kahn, 2008). These events may involve an active, progressive pathology that continues after onset, or a response to a developmental insult(s) that changes with the aging process. Several studies have reported genome-wide expression changes in schizophrenia [reviewed in (Mirnics *et al.*, 2006)], although none have examine the molecular features of aging in this disease.

In our previous studies, we generated gene expression profiles from postmortem human prefrontal cortex (BA46) of 30 normal and 29 schizophrenic subjects ranging from 19 to 81 years of age (Table S1, Supporting Information) (Narayan *et al.*, 2008) using Affymetrix Human Genome U133-Plus 2.0 arrays [see (Narayan *et al.*, 2008) for detailed descriptions of RNA preparations and microarray hybridizations]. We reanalyzed these raw data using linear regression analyses and computed Pearson product-moment correlation coefficients for age against the log2 expression values for 14 439 genes in each subject (Data S1, Supporting Information). After adjusting for multiple statistical testing and the effects of tissue covariables, pH and PMI, the expression of 643 genes in controls and 343 genes in individuals with schizophrenia was found to be significantly correlated with age (Pearson $|r| \geq 0.5$; $P < 0.05$; Table S2, Supporting Information). Surprisingly, there was very little overlap between the two lists of genes, with the expression of only ten genes being correlated with age in both schizophrenic and control subjects. Real-time PCR analysis confirmed expression differences for the top correlated genes (Pearson $|r| \geq 0.8$) in young (19–26 years) vs. older (42–81 years) aged control groups, changes that were not observed in schizophrenic subjects (Fig. 1a).

We next performed the same analysis on microarray expression data from a second cohort of normal subjects ($n = 30$; 26–106 years of age) (Lu *et al.*, 2004), using data freely available on the GEO/NCBI website (record #GDS707), and compared the resulting correlated genes to those obtained from subgroups of our normal population. Using the chi-squared test for independence to compare frequencies of overlapping genes, we found that the expression of a significantly greater number of genes was correlated with age between both normal cohorts compared to those genes correlated with age between normal and schizophrenic cohorts ($n = 130.0 \pm 75$ vs. 6.6 ± 3.33, respectively; $\chi^2 = 139.8–1647$; $P = 0.00$). Thus, the relative similarities among the normal populations indicate that the lack of similarity in age-correlated variation between schizophrenics and normals is not due to general

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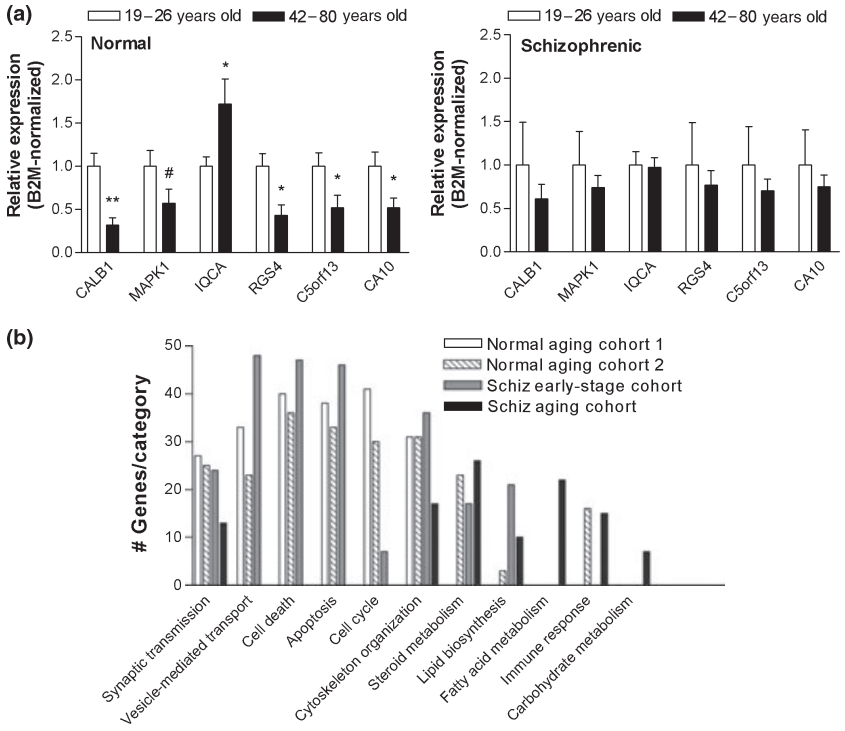

Fig. 1 (a) Real-time PCR analysis of expression levels for the indicated genes in human prefrontal cortical samples (BA46) from normal and schizophrenic individuals. The relative abundance of each gene expression was normalized by beta-2 microglobulin (B2M) and beta-tubulin (TUBB) in young vs. old human samples, respectively. Data are depicted as fold-change of the mean expression level ± SEM (n = 8–12 normal and/or schizophrenic subjects per). Student's t-tests were used to determine significant differences in gene expression levels. Genes are denoted by their official Unigene gene symbol IDs. *Denotes significantly different from control at $P < 0.05$, **$P < 0.01$, two-tailed t test; #denotes significantly different from controls at $P < 0.05$, one-tailed t-test. (b) Gene ontology categories significantly represented in each group of subjects, as indicated. Gene ontology classification was performed using the DAVID database. The numbers of genes in each category is shown on the y-axis.

heterogeneity within the population or between microarray datasets.

'Functions' analyses of our gene lists using The Database for Annotation, Visualization and Integrated Discovery (DAVID) database indicated that the normal aging process was significantly linked to abnormalities in pathways related to synaptic function, cell cycle/DNA damage and apoptosis (Fig. 1b), consistent with previous microarray studies investigating aging in normal humans (Erraji-Benchekroun *et al.*, 2005; Yankner *et al.*, 2008). In contrast, aging in schizophrenia was significantly associated with fatty acid and steroid metabolism, but not with those functions associated with normal aging (Fig. 1b).

The different age-related expression profiles detected in schizophrenic subjects might result from a progressive pathogenic process, a response to pathology or, possibly, drug treatment, considering that a confounding factor in postmortem research on schizophrenia is the unknown effect of antipsychotic drugs, which are known to alter gene expression (Thomas, 2006). Arguments against a strong treatment effect in our data include the facts that the expression of only one of the 343 age-correlated genes was correlated with the patients' recorded drug doses, and that we found no changes in expression of a subset of these genes in the brains of mice treated with haloperidol (2 mg kg^{-1}) or fluphenazine (2.5 mg kg^{-1}) (Fig. S1, Supporting Information), the same drugs with which most of the schizophrenic subjects were treated (Table S1, Supporting Information). However, it remains possible that antipsychotic drug treatment might affect transcriptome profiles in aging schizophrenics.

Given Kraepelin's description of premature dementia in schizophrenia, it is perhaps not surprising that, at disease onset, schizophrenia is associated with a decline in cognition and adaptive functioning, similar to that observed in normal aging. Recent studies have also detected microglia activation, which is associated with normal aging (Miller & Streit, 2007), in recent-onset schizophrenia (van Berckel *et al.*, 2008). Furthermore, normal aging has been linked to alterations in white matter density and volume, gray matter volume decline, cognitive dysfunction and psychotic symptoms (van der Werf *et al.*, 2007; Yankner *et al.*, 2008), which also characterize schizophrenia at first episode (Lieberman, 1999; Steen *et al.*, 2006; Witthaus *et al.*, 2008). Given these commonalities, we hypothesized that early-stage schizophrenia and normal aging might share common molecular underpinnings. We compared transcriptome variation between schizophrenic subjects in early stages of illness (up to 4 years from initial diagnosis; n = 8) vs. matched controls from our previous studies [see Table S3, Supporting Information; (Narayan *et al.*, 2008)] with that generated from linear regression correlation through aging in normal subjects described above. Surprisingly, we found a substantial overlap between these two gene lists: 189 of the genes (29.3%) whose expression levels were correlated with age in normal subjects was concordantly dysregulated in subjects with early-stage schizophrenia compared to age- and sex-matched controls. A further group-wise comparison of young (< 38 years of age) vs. aged normal subjects (> 50 years of age) using ANOVA (Data S1, Supporting Information) revealed 811 differentially expressed genes

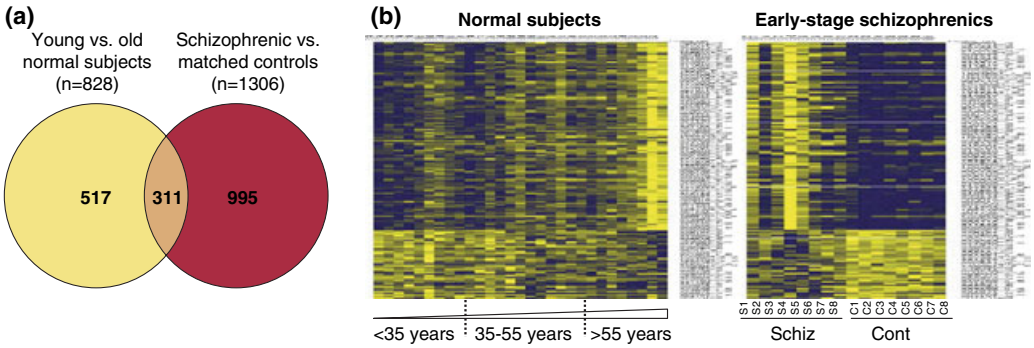

Fig. 2. (a) Overlap of genes differentially expressed in early-stage schizophrenia with those occurring in normal aging ($P < 0.05$). Gene lists used for these comparisons are provided in Table S3. (b) Heatmap visualization of the expression values ($\log_2$-transformed; unclustered) of the overlapping genes shown in (a) in normal subjects throughout aging ($n = 30$ subjects) and in schizophrenic subjects ≤ 4 years from diagnosis [$n = 8$ schizophrenic subjects (S1–S8) and matched controls (C1–C8)]. Each colored pixel represents an individual gene expression value from a single subject. Relative decreases in gene expression are indicated by yellow and increases in expression by blue.

($P < 0.05$; Table S3, Supporting Information), 34.2% of which also significantly varied in subjects with early-stage schizophrenia (Fig. 2a). These overlaps were actually greater than those observed between our two control populations and significantly greater than that predicted from two independent samples ($\chi^2 = 13.9$; $P = 0.0009$). Heatmap depictions of these transcriptome profiles during normal aging and early-stage schizophrenia are shown in Fig. 2b. We also found that pathways/functions associated with early-stage schizophrenia identified by DAVID searches were similar to those related to normal aging and totally different from those associated with aging in schizophrenia (Fig. 1b).

These data demonstrate that the molecular correlates for aging differ between schizophrenic and normal subjects and that normal aging and early-stage schizophrenia share common molecular signatures, suggesting that the onset of schizophrenia anticipates the normal aging process. In addition, we suggest that some symptoms of aging, i.e. dementia and psychosis, might be explained by these common molecular profiles.

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Author contributions

BT and WC performed research and analyzed data; CML performed statistical analyses; BD provided subject samples; JGS and EAT interpreted data and wrote the manuscript.

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Supporting Information

Additional supporting information may be found in the online version of this article:

Appendix S1 Supplementary methods.

Fig. S1 Real-time PCR analysis of expression levels for the indicated genes in cortex of mice treated with haloperidol (2 mg kg^{-1} day^{-1}) or fluphenazine (2.5 mg kg^{-1} day^{-1}).

Table S1 Demographic and array parameter data for all subjects.

Table S2 Complete list of genes whose expression is correlated with age in normal and schizophrenic subjects.

Table S3 Differentially expressed genes in young vs. old normal subjects and early-stage schizophrenic subjects vs. matched controls.

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