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Brain molecular aging, promotion of neurological disease and modulation by Sirtuin5 longevity gene polymorphism

Christin Glorioso [a,b], Sunghee Oh [c], Gaelle Guilloux Douillard [a], Etienne Sibille [a,b,*]

[a] Department of Psychiatry, University of Pittsburgh, Pittsburgh, PA 15213, USA
[b] Center For Neuroscience, University of Pittsburgh, Pittsburgh, PA 15213, USA
[c] Department of Biostatistics, University of Pittsburgh, Pittsburgh, PA 15213, USA

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ABSTRACT

Mechanisms determining characteristic age-of-onset for neurological diseases are largely unknown. Normal brain aging associates with robust and progressive transcriptome changes ("molecular aging"), but the intersection with disease pathways is mostly uncharacterized. Here, using cross-cohort microarray analysis of four human brain areas, we show that neurological disease pathways largely overlap with molecular aging and that subjects carrying a newly-characterized low-expressing polymorphism in a putative longevity gene (Sirtuin5; $SIRT5_{prom2}$) have older brain molecular ages. Specifically, molecular aging was remarkably conserved across cohorts and brain areas, and included numerous developmental and transcription-regulator genes. Neurological disease-associated genes were highly overrepresented within age-related genes and changed almost unanimously in pro-disease directions, together suggesting an underlying genetic "program" of aging that progressively promotes disease. To begin testing this putative pathway, we developed and used an age-biosignature to assess five candidate longevity gene polymorphisms' association with molecular aging rates. Most robustly, aging was accelerated in cingulate, but not amygdala, of subjects carrying a SIRT5 promoter polymorphism (+9 years, $p = 0.004$), in concordance with cingulate-specific decreased SIRT5 expression. This effect was driven by a set of core transcripts (+24 years, $p = 0.0004$), many of which were mitochondrial, including Parkinson's disease genes, PINK-1 and DJ-1/PARK7, hence suggesting that $SIRT5_{prom2}$ may represent a risk factor for mitochondrial dysfunction-related diseases, including Parkinson's, through accelerated molecular aging of disease-related genes. Based on these results we speculate that a "common mechanism" may underlie age-of-onset across several neurological diseases. Confirming this pathway and its regulation by common genetic variants would provide new strategies for predicting, delaying, and treating neurological diseases.

Introduction

Disease-specific ages of onset are core features of many neurological disorders, ranging from late-onset neurodegenerative diseases such as Alzheimer's and Parkinson's diseases (mean age at diagnosis ~70 (Van Den Eeden et al., 2003) and ~80 years (Nussbaum and Ellis, 2003), respectively) to earlier onset psychiatric disorders such as schizophrenia and bipolar disorder (average onset ~25 years) (Tsuang and Tohen, 2002). The mechanism(s) underlying age thresholds and the factors that contribute to individual variability in ages of onset within diseases are largely unknown. Studies have predominantly focused on contrasting disease brains with chronologically age-matched controls, a strategy that may be incomplete, as it is becoming increasingly evident that normal aging rates are an integral aspect and modulator of disease onset and progression. Evidence for this comes from the sheer prevalence of diseases with increasing age, such as Alzheimer's disease (AD), which increases exponentially from age 75 upward reaching nearly 45% by age 95 (Nussbaum and Ellis, 2003). Also, animal models of extended longevity show corresponding delay of age-related diseases, including neurological diseases. For example, in both C. elegans and mice lifespan extension via reduction of insulin/insulin growth factor signaling resulted in delayed proteotoxicity in AD models (Cohen et al., 2006, 2009). Additionally, potential life extension by caloric restriction in primate models has demonstrated delayed brain atrophy (Colman et al., 2009) and Aβ deposition (Qin et al., 2006), and improved functional outcomes in the context of modeled Parkinson's disease (PD) (Maswood et al., 2004). Similarly, rodent studies link inflammation, insulin resistance, and their treatment with Alzheimer's disease pathology burden (Jiang et al., 2008; Yan et al., 2003). Lastly, human genetic and environmental risk factors associated with normal cognitive decline also often associate with age-gated neurological diseases. For example, the APOE4 allele and BDNF val66met alleles associate both with accelerated rates of normal cognitive decline (reviewed in Deary et al. (2004)) and earlier onset of AD and PD (Feher et al., 2009; Li et al., 2004) and epidemiological studies have shown

* Corresponding author. University of Pittsburgh, Department of Psychiatry, 3811 O'Hara Street, BST W1643, Pittsburgh, PA 15213, USA. Fax: +1 412 624 9910.
E-mail address: sibilleel@upmc.edu (E. Sibille).
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that caloric restriction is associated with reduced AD and PD risk (reviewed in Joseph et al. (2009)).

What is occurring during the normal aging of the brain that is required for disease onset? Robust morphological and molecular changes progressively occur in the normal aging brain throughout adulthood and into old age (reviewed in Yankner et al. (2008)). Morphological changes include progressive loss of grey matter density (Resnick et al., 2003), disrupted myelination, and increasing reactive gliosis. These changes reflect dendritic shrinkage, synaptic loss (Morrison and Hof, 1997; Yankner et al., 2008), and thickening glial processes (glial dystrophy) (Conde and Streit, 2006). Within neurons, increased DNA damage and reactive oxygen species, calcium dysregulation, mitochondrial dysfunction and inflammatory processes have been reported (reviewed in Yankner et al. (2008)). Several groups, including our own, have characterized the molecular underpinnings of these changes using human post-mortem brain microarray (Berchtold et al., 2008; Erraji-Benchekroun et al., 2005; Lu et al., 2004). Additionally, studies have shown significant overlap with and anticipation of "normal" brain-aging molecular changes in AD by cross-study microarray analysis (Avramopoulos et al., 2010; Miller et al., 2008). However, no systematic effort has been undertaken to explore the effect of normal molecular aging on disease pathways, which we hypothesize would be substantial and disease-promoting. We further hypothesize, as molecular age accurately predicts chronological age (Erraji-Benchekroun et al., 2005), that individual differences in molecular brain-aging rates may be under genetic control. Specifically, as proof of concept, we tested whether candidate longevity gene variants may associate with rates of molecular brain aging, and more specifically of disease pathway aging.

Here, using microarray analysis of four human brain areas in two cohorts, we show that neurological disease genes are highly age-regulated and change in disease-promoting directions, and that subjects carrying a newly-characterized low-expressing polymorphism in a candidate longevity gene (Sirtuin5; $SIRT5_{prom2}$) have older brain molecular ages, potentially through accelerated decline of mitochondrial function with age. Our results lead us to hypothesize that a common and genetically-controlled mechanism may underlie age-of-onset across several neurological diseases.

Materials and methods

Cohorts and microarrays

We employed two previously described microarray datasets: Cohort 1 (Erraji-Benchekroun et al., 2005) [39 subjects; ages 14–79; prefrontal cortex (PFC) Brodmann area 9 (BA9) and 47 (BA47) samples] and Cohort 2 (Sibille et al., 2009) [36 subjects, ages 23–71; anterior cingulate cortex (ACC) and amygdala samples]. Subject characteristics, dissection protocols, and array controls were described in Erraji-Benchekroun et al. (2005) and Sibille et al. (2009) and are summarized in Supplementary Table 1. All subjects were free of age-related neurological diseases at time of death according to medical records. Pathologist examination of brain tissue revealed no tangles and no or very few plaques, consistent with normal brain aging. Because there were very few subjects with plaques and the standard methodology used by pathologists is only semi-quantitative, we were not able to assess the association of molecular age with extent of normal age-related pathology in either cohort. GC-RMA-extracted data from Affymetrix HU133A (Cohort 1) and HU133Plus2.0 (Cohort 2) arrays were used. Control variables included technical measures (array quality controls, RNA integrity, and post-mortem interval) and subject characteristics (race, gender, and mode of death). All array data are available at our website (http://www.sibille.pitt.edu), including a searchable database for age effects on gene transcript levels in the human brain.

Importantly, both cohorts included subjects diagnosed with major depression (Supplementary Table 1). We have previously shown (and confirmed here) that the gene-expression correlates of depression were of greatly reduced scope compared to the effects of aging. Specifically, in Table S1, we show for both cohorts that the effect sizes of aging are between 184 and 986 times greater than the effect sizes of major depression at the same significance cutoff of $p<0.001$ (aging: 814–1972 transcripts per brain area, depression: 2–6 transcripts per brain area) and 40–50 times greater at the $p<0.01$ cutoff, and that major depression effects do not survive Benjamini–Hochberg control for multiple testing. Moreover, as previously described (Erraji-Benchekroun et al., 2005), major depression was not associated with deviations in molecular ages (Supplemental Fig. S1). So since human brain samples are a limited resource and as the effects of depression are of limited scope and do not associate with altered rates of molecular aging, we have included these subjects in the current analysis in order to increase analytical power (see Discussion).

Defining and validating age-regulated genes

For congruence with the progressive pattern of structural (decreasing grey matter) and functional (cognitive decline) brain-aging changes (Brickman et al., 2006; Resnick et al., 2003), we used best-fit age-regression coefficients to determine significance of age-related gene transcript changes across subjects (Fig. 1a, Supplementary Tables 1 and 2). For each transcript, equations were generated for linear, log, exponential, and power fits of expression level vs. chronological age and the most significant (best-fit) equation was selected (p-values derived from correlation R-values). False discovery rates (FDR) were estimated using Benjamini–Hochberg methodology (Benjamini and Hochberg, 1995). QPCR validation for 42 array-defined age-regulated genes are described in Erraji-Benchekroun et al. (2005) and Sibille et al. (2009) and in online supplements (Supplementary Fig. 2).

Cross-sectional brain-area comparisons

Transcripts with age-regression $p<0.001$ were selected for each brain area, and regression equations were solved for percentile expression changes between 20 and 70 years of age. Directed Pearson correlations (Oh et al., 2009) were performed by correlating these expression changes with transcript levels for the same genes in the other three brain areas.

Age-related biosignatures

Genes were included in the cross-area biosignature if they displayed age-regression $p<0.01$ with age in 3/4 brain areas and $p<0.05$ in the fourth, and if directions of age-regulated changes were concordant in all brain areas. Notably, all but one gene that met the first criteria did not pass the second (HTR2A—both probesets increased with age in amygdala but decreased in cortical areas). If more than one probeset per gene met both criteria, the probeset with the lowest p-value across areas was selected to avoid any gene having a greater weighted influence on molecular age. For ACC and amygdala-specific biosignatures, genes were selected if they had age-regression $p<0.01$ in those areas. Cross-area biosignature genes, cross-area equations, regression R-values, p-values, and magnitude of expression changes are available online (http://www.sibille.pitt.edu/data.html).

Transcriptome functional analyses

Analyses were performed using Ingenuity® version 7.0 and the connectivity map (C-MAP), as described in the respective websites [http://www.ingenuity.com/; http://www.broadinstitute.org/cmap, (Lamb et al., 2006)] and in the supplements.

Molecular ages

Individual predicted molecular were calculated for all age-regulated genes using a leave-one-out approach within ACC or amygdala (Supplemental Fig. S7), as previously described (Erraji-

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Benchekroun et al., 2005). In short, to describe each sample individually in the general aging trend, we have devised a one-number-summary ("Molecular age") for each sample, describing the "predicted age" of the sample when removed from the analysis. For each sample, the remaining database was analyzed for age-related genes using the same correlation-based methods described above,

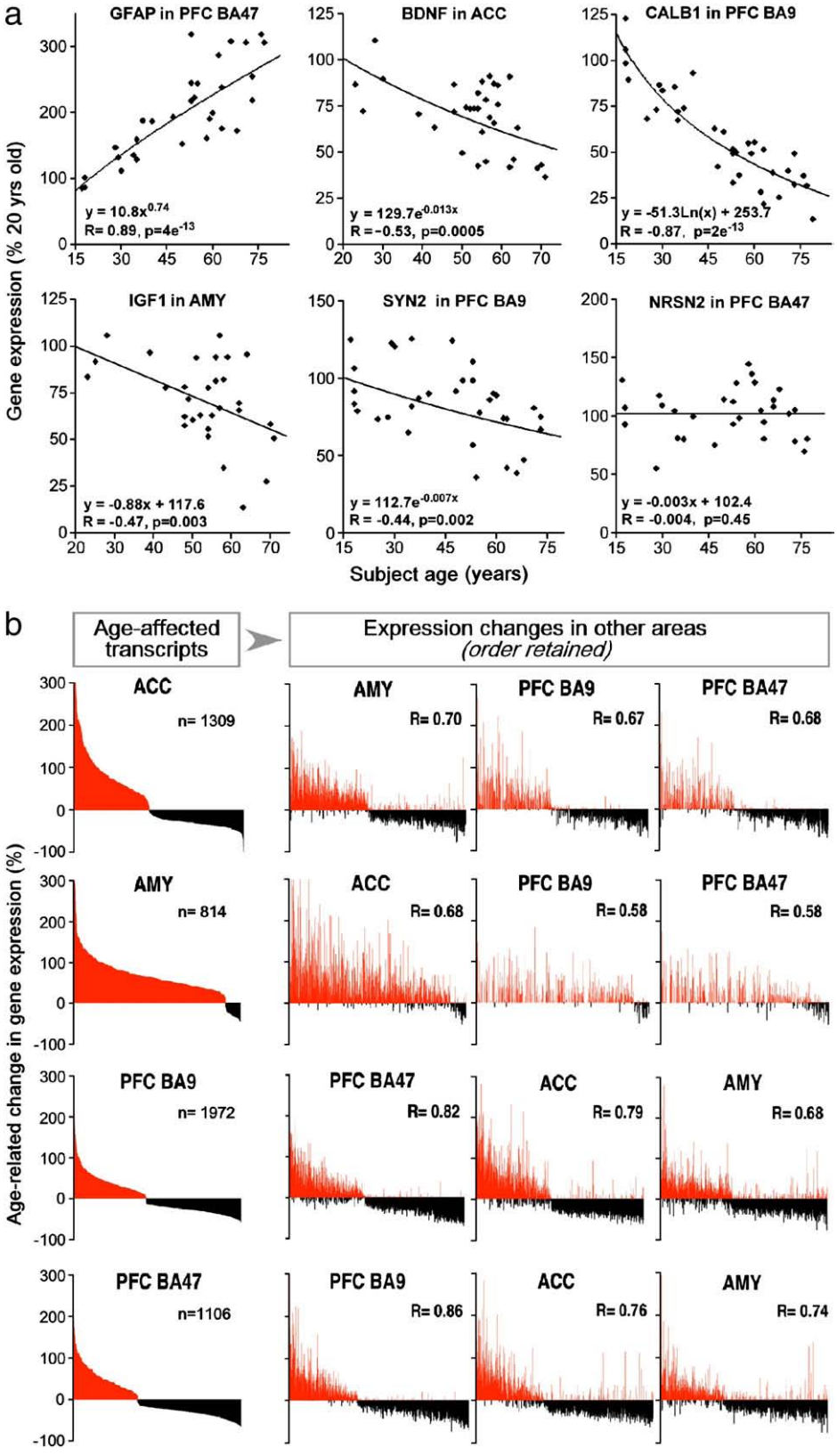

Fig. 1. Conserved molecular aging profiles across human brain areas. a) Representative age-regression gene plots and b) cross-area comparisons of age-regulated gene-expression changes [70–20 years change; ordered from most increased with age (red) to most decreased with age (black)]; n = number of age-regulated genes; R = directed Pearson coefficient. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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controlling the FDR at 0.05. For each selected gene, a best-fit regression analysis with age was performed, and the age for the held-out sample was predicted using the resulting function. Extreme outlier molecular ages (±10 standard deviations from average chronological age) were removed. The resulting gene-wise predicted values were averaged per sample and used to describe the predicted molecular age of each subject.

$SIRT5_{prom2}$ (rs9382222) effects on molecular age

$SIRT5_{prom2}$ is located in a mouse/human conserved region predicted by two separate programs to contain a promoter, TSSG CGG Nucleotide Sequence Analysis (http://www.genomic.sanger.ac.uk/gf/gf.html) and Promoter 2.0 (http://www.cbs.dtu.dk/services/Promoter/) (Supplementary Fig. 6). Cohort 2 subjects were genotyped by sequencing of polymerase chain reaction (PCR) amplified segments of genomic DNA obtained from brain samples. Subjects were 50% C/C and 37% C/T in agreement with Hap-map (http://www.hapmap.org) published frequencies for CEU subjects (Supplementary Table 9). Rare T/T subjects were excluded from analysis because of lack of power. Genotypic differences in all gene transcript levels were calculated using two-tailed Students t-tests in middle-aged cross-sectional groups rigorously matched for chronological age, C/C ($n=12$, average age = 52.1 years, range = 49–63 years) and C/T ($n=11$, average age 52.7 years, range 48–64 years). Similarly significant (although ~10% fewer affected genes) results were obtained using the alternative approach of including all subjects and controlling for age and other parameters by ANOVA.

To assess snp-based group differences, molecular ages were subtracted from chronological ages to assess deviations of molecular from chronological age, thus removing the effect of chronological age. Two-tailed t-tests were performed to obtain p-values associated with difference in total molecular age between genotype-defined groups. A parallel analysis using an ANOVA model yielded similar and significant results, although slightly less robust. This analysis was also performed using only age × snp effect intersection transcripts (Fig. 5b). We refer to these transcripts here as 'intersection transcripts'.

Real-time quantitative PCR (qPCR)

qPCR was performed as previously described (Sibille et al., 2009). Results were calculated as the geometric mean of relative intensities compared to three internal controls (actin, glyceraldehyde-3-phosphate dehydrogenase and cyclophilin).

In situ hybridization (ISH)

T7 and SP6 promoters were incorporated into a 471 base-pair template for the synthesis of SIRT5 transcript probes by PCR, corresponding to bases 851–2282 of the human (GenBank NM_012241.3). Antisense and sense riboprobes were transcribed in the presence of 35S-CTP (Amersham Biosciences, Piscataway, NJ) using T7 and SP6 RNA polymerases, DNase I-digested, and purified (RNeasy mini-columns; Qiagen, Valencia, CA). Three 20 μm sections per subject (total, $n=4$ subjects) were fixed with 4% paraformaldehyde in PBS solution, acetylated with 0.25% acetic anhydrate in 0.1 M triethanolamine/0.9% NaCl for 10 min and dehydrated with a graded alcohol series. The sections were hybridized with 35S-labeled riboprobes (1.0×10^6 cpm/slide) at 56 °C for 16 h, washed, treated with RNAse A, rinsed, and dehydrated through a graded ethanol series, air dried, and exposed to BioMax MR film (Kodak, Rochester, NY) for 5 h. Autoradiographic films were captured, digitized, and analyzed with a Microcomputer Imaging Device (MCID; Imaging Research Inc., London, Ontario, Canada). Adjacent sections stained with cresyl violet were superimposed onto autoradiographic images to draw contours of the full cortical thickness perpendicular to the pial surface. Optical density measures within each sampled area were calibrated to radioactive carbon-14 standards (ARC Inc., St. Louis, MO) exposed on the same film, and expressed as nanocuries per gram (nCi/g) of tissue.

Results

Molecular aging is conserved across cohorts and brain areas

We employed two previously described microarray datasets to investigate the extent and conservation of altered gene expression with age in the human brain (see Materials and methods): Cohort 1 [39 subjects; ages 14–79; prefrontal cortex (PFC) Brodmann area 9 (BA9) and 47 (BA47) samples] and Cohort 2 [36 subjects, ages 23–71; anterior cingulate cortex (ACC) and amygdala samples]. At $p<0.001$, 814–1972 transcripts were age-regulated in each brain area with 1–4% estimated FDR (Supplementary Table 1). Array data were previously validated by high correlation with independent quantitative PCR (qPCR) results ($n=42$ genes, $R=0.72$, $p=10^{-10}$, Supplementary Fig. 1) and by known age-regulated genes changing in predicted directions, including up-regulated reactive gliosis markers (GFAP), and down-regulated growth factors (BDNF and IGF-1), synaptic markers (SYN2) and calcium homeostasis genes (CALB-1) (Berchtold et al., 2008; Lu et al., 2004) (Fig. 1a). Expression changes did not reflect age-related changes in cell number, as many neuronal-specific transcripts were unchanged with age [NRSN2 (Nakanishi et al., 2006); Fig. 1a], consistent with stereological studies demonstrating minimal neuronal loss during normal aging (Morrison and Hof, 1997).

Molecular aging was remarkably conserved across cohorts and brain areas ($p<10^{-10}$, Fig. 1b). Gross area-specific differences were only observed in amygdala, with fewer age down-regulated transcripts (Fig. 1b, $n=87$) compared to cortical areas ($n=684$–1133). We have previously shown that down and up-regulated changes are predominantly of neuronal and glial origin respectively (Erraji-Benchekroun et al., 2005; Sibille et al., 2008) (Supplementary Table 3); thus the fewer observed down-regulated neuronally-enriched changes still correlated with changes in other brain areas, but were "noisier" (higher p-values, Supplementary Tables 3–5), consistent with structural MRI studies reporting robust cortical and more variable amygdala age-related grey matter losses (Good et al., 2001).

A novel age-related biosignature predicts chronological age, contains development- and neurological disease-related genes, and is potentially regulated by cell-cycle and neurotransmitter-modulatory drugs

To assess cross-sectional rates of molecular aging, we developed a brain- and age-related biosignature, based on conserved changes across areas ($n=356$ genes). Transcript levels were converted into "molecular ages" using cross-area age-regression equations, which were averaged to generate a single molecular age per subject per brain area, using a leave-one-out approach to avoid circularity. The biosignature was highly predictive of subject age ($p<10^{-16}$, Fig. 2a), confirming its utility as a quantitative assay and the cross-area robustness of age-related transcript changes. Savva et al. (2010) have shown that correlation between age biological changes and age may be lower above 75 years of age in subjects with dementia (Savva et al., 2010). Here included subjects were free of overt neurodegenerative disease (see Materials and methods), so we did not place any cap on predicted molecular ages, hence we may have slightly underestimated the true correlation between chronological and predicated ages (Fig. 2a).

Using large-scale hand-annotated literature information, Ingenuity® biological pathway analysis identified the expected categories of known age-related changes in the biosignature (cell morphology, signaling, immune response, vascular function, cell death, DNA repair and protein modification) (Supplementary Tables 6 and 7).

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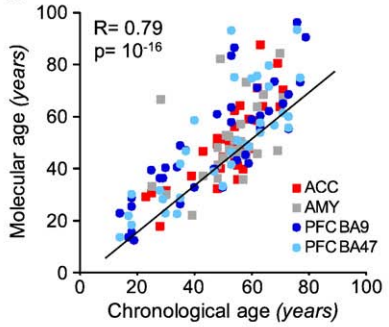

b Epigenetic regulators & Transcription factors

Gene	N	G	B	E(%)	P-val
Histone cluster 1, H2bk			✓	-34	1E-16
Down-regulator of Transcription 1			✓	-40	5E-11
Methyl CpG Binding Protein 2			✓	+24	2E-10
Mitochondrial ribosomal protein S12	✓			-39	2E-10
Methyl-CpG Binding Domain 1	✓			-18	1E-06
Chromatin Modifying Protein 1B	✓			-18	4E-06
Forkhead Box O1		✓		+42	2E-06
H3 Histone, family 3A/3B		✓		+73	2E-16
Transcription Factor EB		✓		+66	6E-09
CREB-regulated Transcription Coactivator		✓		+39	3E-10
Jumonji, AT-rich Interactive Domain 1A		✓		+42	1E-08
HMG-box Transcription Factor 1		✓		+42	1E-11

c C-Map Candidate Brain Aging Drugs

Drug	Mechanism	P-val
Anti-aging		
GW-8510	CDK2 inhibitor; neuroprotective	1E-04
a-estradiol	Estrogen enantiomer; neuroprotective	1E-04
Urapidil	Antihypertensive, a1 adrenergic antagonist, a2 adrenergic agonist, 5HT1A agonist; neroprotective	2E-04
Alsterpaullone	Inhibitor of CDK2, CDK1/Cyclin B, CDK5/p25, GSK-3ß, Tau phosphorylation; neuroprotective	5E-04
Skimmianine	Furoquinoline alkaloid (plant extract) used in folk medicine; potentially anti-inflammatory, anti-tumorigenic, MAO inhibitor	6E-04
H-7	PKC inhibitor, decreases calcium current, alters astrocyte morphology	7E-04
Niacin (vitamin B3)	Anti-inflammatory properties; used to treat hypercholesterolemia, arteriosclerosis, and cardiovascular disease	0.005
Biotin (vitamin B7)	Regulates insulin secretion; therapeutic efficacy in diabetes	0.009
Pro-aging		
Wortmannin	Inhibitor of PI3-K cell survival pathway	1E-06
Benzamil	Na/Ca exchange blocker, inhibitor of NGF mediated neurite outgrowth	5E-04

Fig. 2. Age-related biosignature. a) Predictability of subject ages and b) proposed regulatory genes. N (neuronally-enriched), G (glial-enriched), B (similarly expressed in neurons and glia), and E (expression 70–20 years, all brain areas). c) Biosignature-predicted age-modulatory drugs.

Additionally, nervous system development was a top category (70 associated genes, Supplementary Table 6). Notably, this identified functional group included epigenetic regulators, transcription factors, and histones, consistent with potential roles in regulating some of the observed transcript changes, and suggesting the presence of a putative age-related transcriptional program (Fig. 2b). Importantly, neurological disease was a top category (115 associated genes), supporting our hypothesis of disease promotion by normal aging (Supplementary Tables 6 and 7).

We further characterized the age-related biosignature using the microarray drug-matching program, C-MAP (Lamb et al., 2006), by identifying drugs causing transcriptional changes in cell culture inversely correlating with our biosignature (candidate anti-aging drugs). As an internal validation, C-MAP identified known anti-aging and neuroprotective agents, such as α-estradiol and GW-8510, an inhibitor of neuronal apoptosis (Fig. 2c). Interestingly, results pointed to regulatory roles for cell-cycle proteins and neurotransmitters as candidate anti-aging drugs, as two of the top six drugs were cyclin-dependent-kinase inhibitors and two were monoaminergic modulators (Fig. 2c).

Neurological disease-related genes are overrepresented amongst age-regulated genes and change in pro-disease directions

To characterize the extent of overlap between age and disease pathways, we selected a wider gene group ($n = 3935$) not restricted by significance in all brain areas ($p<0.001$ in one area, or $p<0.01$ in two). Again, neurological disease was a top Ingenuity®-identified functional category, comprising 34% of age-regulated genes (Fig. 3a, Supplementary Fig. 3). Consistent with our hypothesis, the top indentified diseases, Alzheimer's, Parkinson's, Huntington's, amyotrophic lateral sclerosis, schizophrenia and bipolar disorder were all common neurological diseases with well-defined ages of onset (Supplementary Fig. 4). Conversely, disease-associated genes represented only 4% of non-age-regulated genes ($p>0.05$ in all areas), and neurological disease fell to the 44th functional category with no specific diseases represented (Fig. 3a, Supplementary Fig. 5). Furthermore, investigations into a subset of genes with well-established disease-associations revealed that expression changes were almost unanimously (32/33) in disease-promoting directions (Fig. 3b, Table 1, Supplementary Table 8). Examples of age-regulated plots for specific disease-related gene are shown in Fig. 3b. Note the discrepancy in rates observed across brain regions for some genes. For instance, clusterin (CLU), an Alzheimer-related gene displayed greatest age-related increase in ACC (red), where neuregulin (NGG-1), a schizophrenia-related gene, showed lowest age-related down-regulation in BA9 (dark blue) (Fig. 3b), together providing potential mechanisms for region-specific onset of pathological symptoms.

$SIRT5_{prom2}$ associates with decreased SIRT5 expression and accelerated molecular aging, particularly of mitochondrial-localized proteins, in a brain-area-specific manner

We next hypothesized that longevity genes may regulate brain aging and that polymorphisms in these genes may influence gene sets involved in risk for disease. We assessed 5 polymorphisms in three candidate longevity genes (Supplementary Fig. 6, Table 9), but focus the remainder of this study on a $SIRT5_{prom2}$ single nucleotide polymorphism (snp), as it was associated with the largest and most statistically robust effects on molecular aging (Supplementary Table 10). We chose SIRT5 due to the increasing role of the sirtuin gene family in neurodegenerative disease (Gan and Mucke, 2008) and due to our previous observation of altered Sirt5 expression in *htr1b*^{KO} mouse cortex, a mouse model with anticipated brain aging (Sibille et

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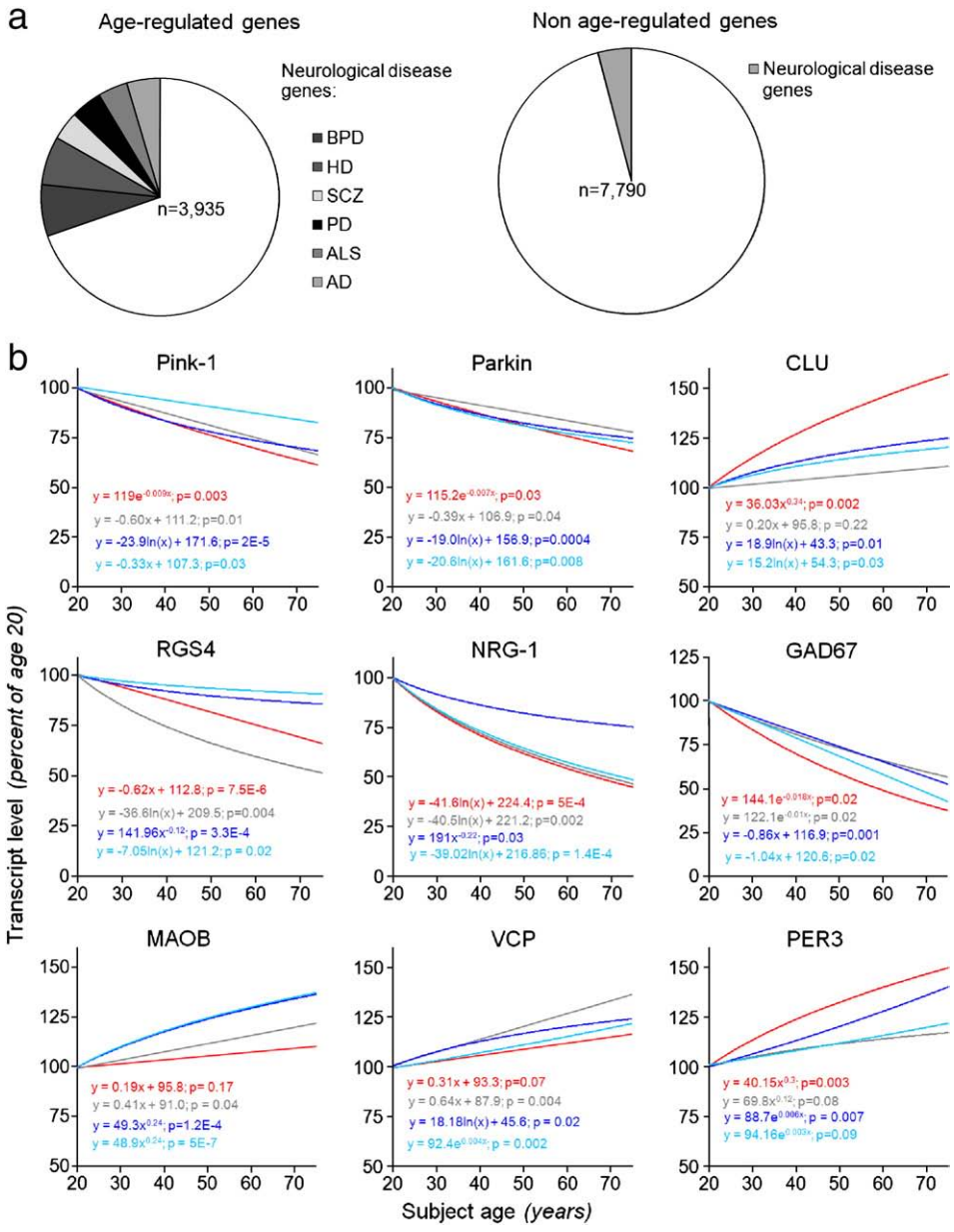

Fig. 3. Molecular aging and neurological diseases. a) Percentage of age and non age-regulated genes identified as neurological disease-related by Ingenuity. Left) 34% (n = 1098) of age-regulated genes were neurological disease-related, including AD (n = 185), PD (n = 170), HD (n = 267), ALS (n = 164), SCZ (n = 161), and BPD associated genes (n = 285). Right) 4% (n = 321) of non-age-regulated genes were neurological disease-related with no specific diseases identified. b) Example plots of age-regulated disease-related genes. Trend lines are best-fit regression lines for ACC (red), amygdala (grey), PFC BA9 (dark blue), and PFC BA47 (light blue) with color-coded equations and corresponding regression p-values. AD, Alzheimer's disease; PD, Parkinson's disease; HD, Huntington's disease; ALS, amyotrophic lateral sclerosis; SCZ, schizophrenia; BPD, bipolar disorder.

al., 2007). We identified the $SIRT5_{prom2}$ as an snp of interest, due to its location in a mouse/human conserved region predicted by two separate programs to contain a promoter region (Supplementary Fig. 6). We also concentrated on Cohort 2 subjects, for which genetic material was available.

Because little is known about the layer-specific localization of SIRT5 in the human brain we performed in situ hybridization in ACC. We show that SIRT5 transcripts are expressed in all cortical layers, with a sharp peak corresponding to layer II (Fig. 4), suggesting a role in local processing of thalamic sensory inputs and projection to deep layers (Wu et al., 2009). We next show by qPCR that the $SIRT5_{prom2}$ polymorphism associates with a 45–55% decrease in expression in SIRT5 transcript in ACC, affecting the two variants of that gene (Fig. 5a). SIRT5 itself did not display age-regulated expression levels (age-regression p = 0.45), thus genotypic differences in expression were present at all ages. No $SIRT5_{prom2}$ genotype effect on SIRT5 expression was observed in amygdala (Supplementary Fig. 8), suggesting a brain-region-specific effect of $SIRT5_{prom2}$. SIRT5 C/C (low-expresser) allele carrier subjects had significantly older ACC molecular ages (+8.6 years, p = 0.003, Fig. 5b) compared to C/T carriers (Supplementary Fig. 7). The observed difference was not due to residual age effect, as the C/C and C/T allele carrier cohorts were rigorously matched for chronological age. Instead, the difference resulted from apparent accelerated ACC molecular aging rates in C/C carriers (increased molecular vs. chronological age slope, Fig. 5b). Using an amygdala-specific biosignature (Supplementary Fig. 7), we show that $SIRT5_{prom2}$ was not associated with altered amygdala molecular aging, consistent with the fact that the $SIRT5_{prom2}$ was not associated with altered SIRT5 level in that brain region (Supplementary Table 10).

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Table 1
Disease-gene age-regulation. Agreement of directions between disease-related and age-regulated (age 70–age 20) gene-expression changes. ↓ = decreased mRNA/protein levels reportedly pro-disease; ↑ = increased mRNA/protein levels reportedly pro-disease; u = unknown/unclear reports of directionality in disease (references in Supplementary Table 8); n.s. = non-significant ($p>0.05$) change with age.

	Direction of change in disease						Change with age (%) (p-val)			
Disease~associated gene _ symbol	AD	PD	HD	ASL	SZC	BDP	ACC	AMY	PFC BA9	PFC
Amyloid beta precursor protein binding-1_Fe65	u						-17.4 (0.005)	n.s.	-25.9 (1.2E-5)	n.s.
Amyloid beta precursor protein binding- 2_APPB2/PAT1	↑						+18.1 (0.02)	+10.1 (0.04)	+22.1 (0.0003)	n.s.
Amyloid precursor-like protein 2_APLP2	↓						-25.4 (0.009)	n.s.	-31.1 (0.0001)	-30.5 (0.008)
Clusterin/apolipoprotein-J_CLU	↑		↑	↑			+80.5 (0.0004)	+29.3 (0.02)	+75.8 (1.3E-7)	+54.8 (5.8E-8)
Monoamine oxidase B_MAOB	↑	↑	↑	↑			n.s.	+20.5 (0.42)	+34.2 (0.00006)	+34.9 (8.5E-7)
Microtubule-associated protein tau_MAPT	↑	↑					-34.9 (0.009)	n.s.	-28.7 (2.9E-6)	n.s.
α-Synuclein_α-syn	↓	u					-32.6 (8.8E-5)	-39.4 (0.03)	-19.7 (1.7E-6)	-20.3 (0.001)
Parkinson disease-2_Parkin	u	↓					-29.5 (0.02)	-19.4 (0.04)	-23.9 (0.0003)	-26.0 (0.009)
Parkinson disease-5_UCHL1		↓					-27.2 (0.001)	-24.6 (0.02)	-14.9 (0.002)	n.s.
Parkinson disease-6_PINK-1		↓					-36.2 (0.003)	-29.9 (0.009)	-29.8 (6.3E-6)	-15.7 (0.03)
Parkinson disease-7_DJ-1		↓					-25.9 (0.0006)	-15.3 (0.02)	n.s.	n.s.
Parkinson disease-13_HTRA2		↓					-27.4 (0.002)	n.s.	-9.5 (0.04)	-25.6 (0.0006)
Huntingtin_HD			↓				n.s.	n.s.	-22.9 (0.0005)	n.s.
Valosin-containing protein_VCP			↑				n.s.	+32.5 (0.003)	+22.9 (0.001)	+22.9 (0.002)
Mitochondrial complex 1 subunit_NDUFB5			↓				-22.0 (0.001)	-28.9 (0.009)	n.s.	n.s.
Mitochondrial complex 1 subunit_NDUSF2			↓				-33.0 (0.0003)	n.s.	-16.8 (0.002)	n.s.
Mitochondrial complex 1 subunit_NDUSF3			↓				-24.4 (6.4E-5)	-22.1 (0.05)	-15.4 (0.01)	-13.9 (0.005)
Mitochondrial complex 1 subunit_NDUSF3			↓				-17.9 (0.0007)	n.s.	n.s.	n.s.
Mitochondrial complex 4 subunit_COX7B			↓				-22.7 (0.0004)	-27.2 (0.004)	n.s.	n.s.
Cyclin-dependent Kinase-5_CDK5	↓		↓				-35.3 (4E-5)	n.s.	-30.9 (1.9E-8)	-25.6 (0.0002)
NF-kappa B_NF-kB	↑	↑	↑	↑		↑	+16.2 (0.03)	n.s.	+24.0 (0.0001)	+15.1 (0.01)
Manganese superoxide dismutase_SOD2	↓			↓	↓		n.s.	n.s.	-50.3 (0.0007)	n.s.
Cholecystokinin_CCK	↓	↓			↓		-33.8 (0.002)	-29.7 (0.03)	-18.1 (0.01)	n.s.
Neuropeptide-Y_NPY	↓	u			↓	↓	-33.8 (0.02)	-41.7 (0.008)	-34.1 (0.003)	n.s.
Cannabanoid receptor-1_CB1	↓	u	↓		↓		n.s.	n.s.	-45.7 (2.6E-10)	-39.4 (0.002)
Parvalbumin_PVALB	↓	u			↓	↓	-58.6 (0.001)	n.s.	n.s.	-34.5 (0.02)
Glutamate decarboxylase 1_GAD67		↓			↓	↓	-59.3 (0.02)	-39.3 (0.02)	-43.2 (0.0009)	-51.9 (0.02)
GABA transaminase_GABA-T	u				↑		+25.3 (0.04)	n.s.	+28.4 (0.0003)	n.s.
Brain-derived neurotrophic factor_BDNF	↓	↓	↓		↓	↓	-45.1 (0.0005)	n.s.	-39.8 (8.9E-6)	-41.8 (3.4E-5)
Serotonin 2A receptor_HTR2A	↓				↓	↓	-40.8 (0.0007)	+64.9 (0.04)	-39.4 (0.0001)	-46.3 (0.0008)
Serotonin 5A receptor_HTR5A					u	u	-39.3 (0.0007)	-32.9 (0.00005)	-33.2 (0.0001)	-34.3 (0.05)
Somatostatin_SST	↓	↓	↓		↓		-45.0 (0.0001)	-61.4 (0.01)	-57.3 (5.4E-6)	-39.4 (0.001)
Regulator of G-protein signaling-4_RGS4	↓		↓		↓	u	-43.5 (0.008)	-75.3 (0.006)	-44.7 (2.0E-8)	-57.5 (2.1E-5)
Reelin_RELN	u	u			↓	↓	-33.0 (0.02)	n.s.	n.s.	-38.1 (0.0002)
Neuregulin-1_NRG1	u				u	u	-52.3 (0.0003)	-50.7 (0.001)	-23.7 (0.03)	-48.9 (0.0002)
Dopamine receptor D1_DRD1	u		↓		u	u	-50.3 (0.008)	n.s.	-33.7 (0.001)	-48.7 (0.006)
GABA receptor, alpha-5 subunit_GABRA5			↓		u	u	-48.3 (0.03)	-59.4 (0.02)	-67.0 (8.3E-10)	-58.9 (0.0003)
Period homolog-3_PER3					u	u	+46.7 (0.002)	n.s.	+35.0 (0.004)	n.s.
Aryl hydrocarbon receptor nuclear translocator-like_BMAL1					u	u	-37.0 (0.005)	n.s.	-44.5 (1.4E-5)	-59.3 (1.4E-5)

We next investigated whether $SIRT5_{prom2}$'s correlation with older molecular ages was global or potentially driven by a subset of genes. We determined the significance of $SIRT5_{prom2}$ genotype association with transcript changes for all other genes in well age-matched subgroups, as an exploratory approach for potential indirect $SIRT5_{prom2}$-mediated effects (Supplementary Fig. S10). $SIRT5_{prom2}$ associated ($p<0.01$) with altered levels for 972 transcripts, including 231 age-regulated transcripts (Fig. 5b). These latter transcript changes almost unanimously (98%) associated with older molecular ages in SIRT5-C/C carriers. Indeed, based on these "core" snp-by-age intersection transcripts, subjects carrying the C/C allele were on average 24.1 molecular years older than C/T carriers ($p=0.0004$, Fig. 5b). We conjecture that these core transcripts represent proximal effectors in SIRT5's putative modulation of age-related expression changes.

These predominantly (74%) neuronally-enriched transcripts included potential brain-aging regulators, transcription factors (GTF3A and TCF7L2), Histone 3 (H3F3A/3B), Chromatin Modifying Protein 2A (CHMP2A), and CDK5 (Supplementary Table 11). Considering that the SIRT5 protein is localized to the mitochondrion (Gan and Mucke, 2008; Nakagawa et al., 2009), it was striking that many of these core transcripts coded for mitochondrial-localized proteins, including numerous components of the electron transport chain (Fig. 5c). The top two identified canonical pathways were mitochondrial dysfunction and oxidative phosphorylation, and the top functional categories —genetic and neurological diseases—were predominated by two diseases linked to mitochondrial dysfunction: Parkinson's (9 associated genes) and Huntington's (22 associated genes) (Fig. 5c, Supplementary Figs. 9–11). Most directly, $SIRT5_{prom2}$ genotype accounted for all age-related declines in expression of the familial Parkinson's genes, PINK-1 and DJ-1/PARK7 (Fig. 5d; qPCR-validated, Supplementary Fig. 12). People with loss of expression/function mutations in these genes develop early onset Parkinson's (Schapira, 2008). Together, these findings suggest that $SIRT5_{prom2}$ may represent a novel indirect risk factor for mitochondria-related diseases, potentially including Parkinson's and Huntington's diseases.

Discussion

Here we investigated the molecular correlates of "normal" human brain aging by microarray analysis in two cohorts and four brain areas, focusing on the overlap of aging and disease pathways, and then tested whether subject molecular brain-aging rates associated with several candidate longevity gene polymorphisms. We show that molecular brain aging is highly selective and consistent across cohorts, contains many transcriptional-regulators, and pushes disease-related genes in disease-promoting directions, and that rates of this promotion are differentially associated with a genetic variant ($SIRT5_{prom2}$) in a modular and brain-area-specific manner. Based on

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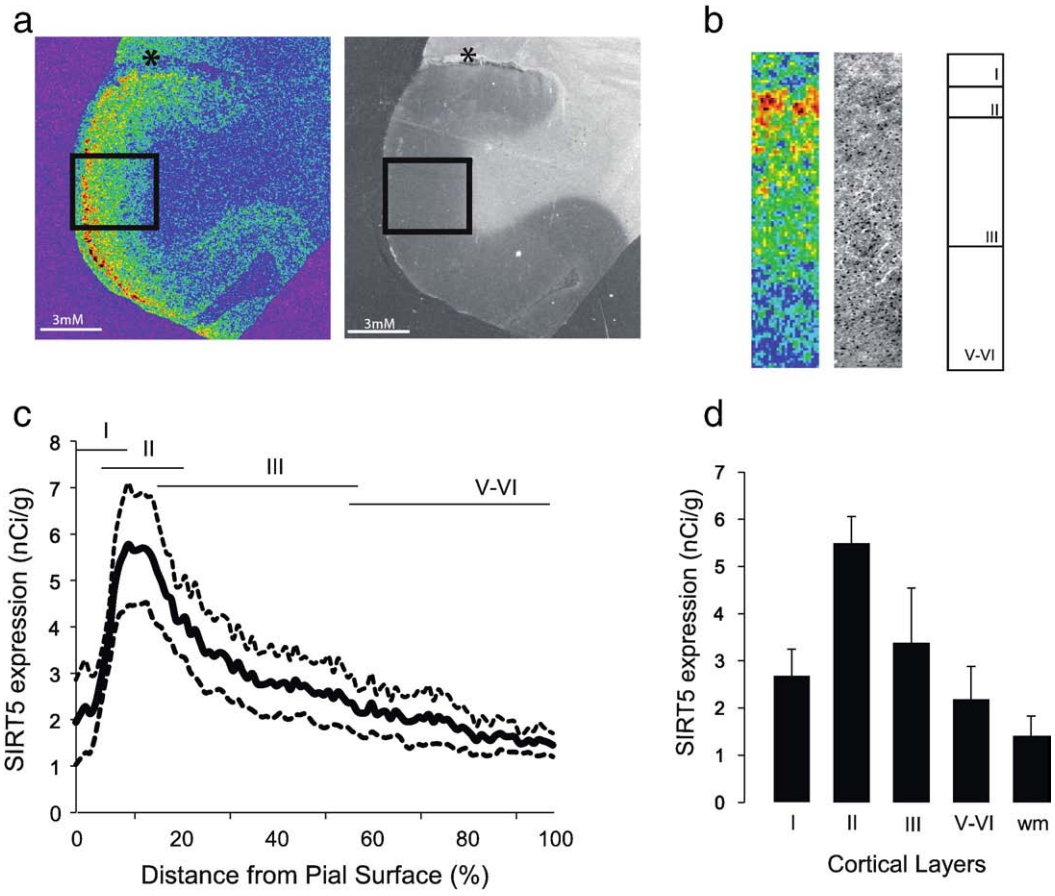

Fig. 4. SIRT5 mRNA expression in ACC. In situ hybridization for Sirtuin5 transcript 1 mRNA expression. a) Representative picture of SIRT5 in situ hybridization pattern in ACC (left), Nissl bodies staining in an area of sampling corresponding to subgenual ACC (right). *, indicates corpus callosum. b) Cortical layer delineation on ISH autoradiograph (left), based on adjacent staining of Nissl bodies (Middle). Layer IV is absent in ACC. c) Average profile of SIRT5 expression indicated by bold line (± sem; indicated by dashed lines) as a function of distance from the apical surface, based on a series of cortical traverse extending from the surface to the white matter, expressed in relative percentage of cortical thickness (4 subjects total; 3 section per subject;). d) Layer-specific quantification, based on Nissl staining and reported layer delineation in ACC. Palomero-Gallagher, 2008.

these results we speculate that molecular brain aging may be a genetically-controlled transcriptional program, generally responsible for the normal brain-aging requirement for neurological disease onset. However, testing of this hypothesis is beyond the scope of the current study and will require further experimentation on a variety of fronts.

Expanding from our prior report (Erraji-Benchekroun et al., 2005), we demonstrate high brain-area and cross-cohort correlation of molecular brain aging ($p<10^{-7}$ between any two areas), selectively affecting ~5–10% of all detected genes. This is fairly remarkable given the historic lack of consistency between microarray findings, especially since these cohorts come from different University studies using different methodologies, including microarray platforms. We interpret this high reproducibility as evidence of the large, specific, and robust phenotype of molecular brain aging, consistent with a biological program. Also consistent with studies in model systems (Chen et al., 2009; Kawahara et al., 2009; Kim et al., 1999; Lin et al., 2001) was the presence of many affected regulators of gene expression (transcription factors, histones, and methylases), potentially regulating cascades of downstream gene-expression changes. These potential mediators offer biologically relevant entry points to molecular dissection of this putative genetic program in model organisms or neuronal culture systems. Moreover, the characterization of an age-derived molecular biosignature predictive of chronological age, pointed towards cell-cycle and neurotransmitter-modulating drugs as potential aging-regulators, offering insight into how age-related changes may be globally regulated.

A notable feature of this molecular signature of aging is a large over-representation of neurological-related genes almost unanimously affected during aging in disease-promoting directions, supporting that pathways to several neurological diseases may be intrinsic aspects of normal aging. Furthermore, the top six represented diseases (Alzheimer's, Parkinson's, amyotrophic lateral sclerosis, Huntington's, bipolar disorder and schizophrenia) unbiasedly identified by Ingenuity pathway analysis, were not only all neurological, but all age-gated. This is consistent with two reports showing significant overlap between microarray changes associated with Alzheimer's and normal aging in human brain (Avramopoulos et al., 2010; Miller et al., 2008). Particularly, Miller et al. (2008) showed that there is a significant overlap between AD-related changes in hippocampus and the normal age-related changes previously reported in PFC (Lu et al., 2004), despite differences in brain areas and methodologies in the compared studies (Miller et al., 2008). For instance, our findings implicating mitochondrial-related genes in aging and disease extend the findings by Miller et al. (2008) of a mitochondrial-enriched gene module at the intersection of normal aging and AD. Similarly, the robust and consistent down-regulation of CDK5 observed here (Table 1), parallels the identification of the same gene as a key hub gene in a shared age and AD gene network in that study (Miller et al., 2008), hence providing a complementary validation in independent datasets, and suggesting CDK5 as a key driver of aging and AD. Avramopoulos et al. (2010) found a more extensive overlap between AD-related changes in temporal cortex and those PFC aging changes,

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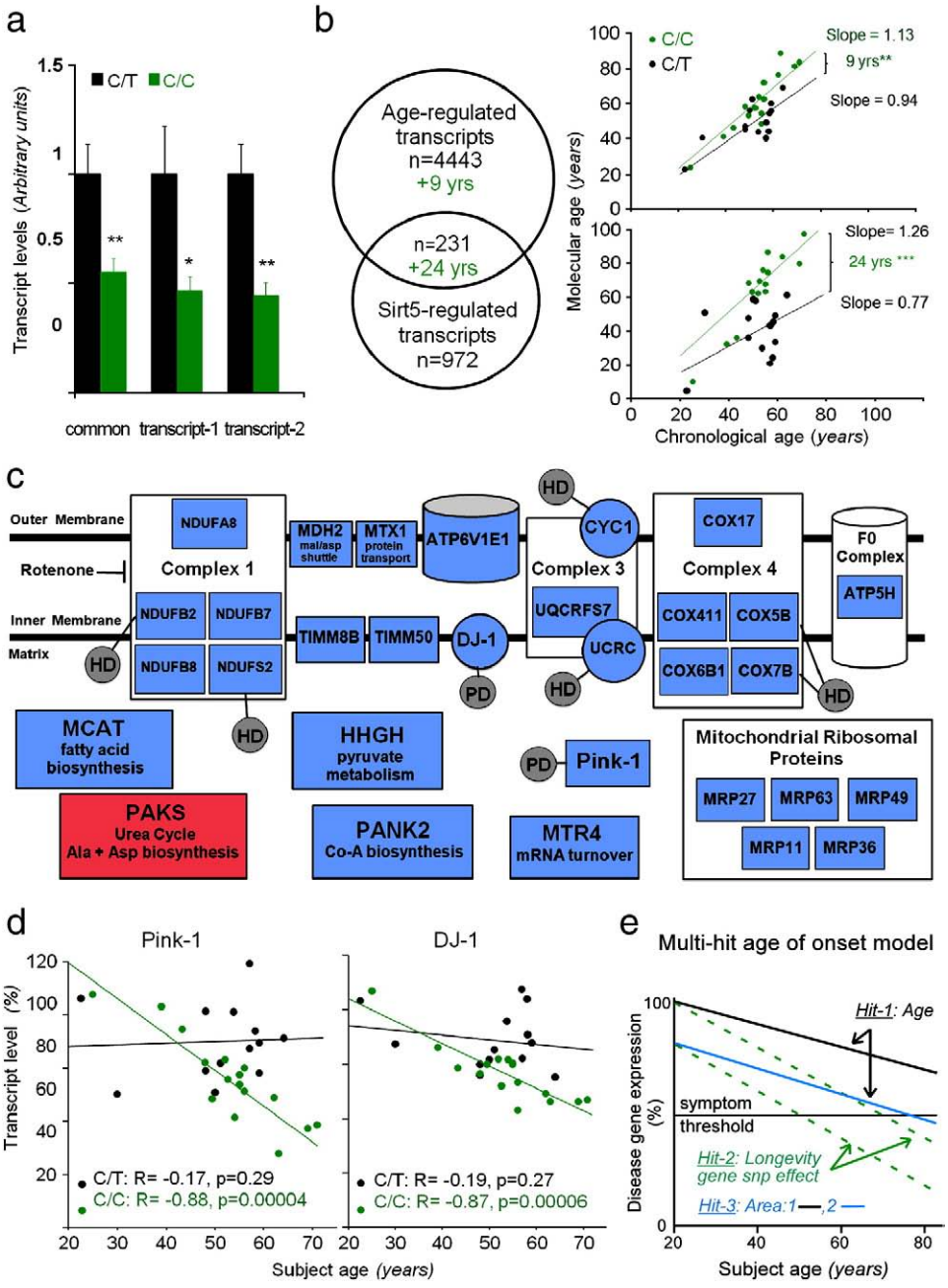

Fig. 5. SIRT5$_{prom2}$ effects on ACC molecular aging. a) SIRT5 expression in ACC by prom2 genotype. b) Venn diagram (ACC) of age ($p<0.01$) and SIRT5$_{prom2}$ ($p<0.01$) associated transcripts; (number yr) = average number of molecular years greater in C/C subjects than C/T (left); molecular ages of subjects by SIRT5$_{prom2}$ genotype (top-right) based on all age-regulated transcripts and core transcripts (bottom-right). c) Schematic of mitochondrial age-regulated genes with accelerated age-trajectories in SIRT5-low-expresser subjects; blue are age down-regulated transcripts; red are age-upregulated transcripts; grey circles identify HD or PD-associated genes. d) Representative core transcript age-regressions (PD genes) by SIRT5$_{prom2}$ genotype. e) Multi-hit model of age-of-onset. *Hit 1*: The expression of a disease-related gene is affected during aging in disease-promoting direction; *Hit 2*: this "rate" of age-regulated changes is accelerated in subjects carrying "risk alleles" for age-modulatory gene polymorphisms (i.e. SIRT5); *Hit 3*: different brain regions have different baseline expression for disease-related genes, hence starting closer to a theoretical threshold of onset of symptoms. These three hits converge to lower the age at which decreased expression reaches a critical theoretical threshold for symptom or disease onset. Conversely, protective factors (genetic and/or environmental) would delay onset. A similar mechanism would occur for age upregulated disease-related genes.

and furthermore found that many normal age-related changes were significantly exaggerated in AD brains vs. chronologically age-matched controls (Avramopoulos et al., 2010). These studies are consistent with our results, but did not specifically test the hypothesis that AD brains have overall older molecular ages than their chronologically age-matched controls and were also limited by incomplete overlap between probesets across the compared array platforms with more limited gene coverage (Lu et al., 2004). We extend these findings to several other diseases using a different approach, showing that hundreds of genes that have been previously associated with AD as well as five other age-gated neurological diseases are robustly affected by age, and change almost unanimously in pro-disease directions. Based on this concordance of age- and disease-related gene-expression correlates, we conjecture that rates of molecular aging of disease-related genes may determine at what age (and therefore if) highly prevalent diseases such as Alzheimer's occur, and at what age less prevalent diseases (Huntington's and Parkinson's) may occur in the context of additional genetic/

environmental hits (Fig. 5e). Further supporting this gene-expression level "gating" model of age-of-onset, promoter polymorphisms affecting disease-gene expression, such as Parkin for instance, associate not only with disease risk, but also with younger disease onset (Sutherland et al., 2007). Thus we hypothesize that people with older molecular brain ages with respect to their chronological ages, and especially accelerated age-related changes in disease-related genes may demonstrate earlier onset of and greater risk for developing these diseases. From a broader evolutionary perspective, we speculate that age-related diseases may stem from partial mutations/polymorphisms in the most age-vulnerable genes, which only become phenotypically expressed in the context of age-regulated disease-promoting expression changes, hence allowing their evolutionary conservation, since they are not embryonically lethal or selected against during the reproductive window. It would follow that age-vulnerable genes may be good candidates for as of yet unidentified disease-related genes.

On a related note, the large representation of age-affected schizophrenia and bipolar-related genes is intriguing, as while these diseases are age-gated, their onsets occur at younger ages and they are often considered development-related diseases. Here, as developmental genes were a top represented age-regulated category, our results support the concept that aging and development may share overlapping pathways, or alternatively, that aging may extend from development. This makes conceptual sense, as synaptic pruning for instance is beneficial during development, but related mechanisms may lead to deleterious synaptic losses during aging. Moreover, similar to our observations during adult and late-life aging, changes in the rates of these developmental processes and associated genes can lead to altered brain connectivity or subtle changes in synaptic connections, as observed in bipolar and schizophrenia. This is consistent with the recent study by Colantuoni et al. (2008) showing robust changes in transcript levels for schizophrenia-related genes during development and early adulthood.

An apparent exception to these observations of age-by-neurological disease interaction is the absence of major depression-related functional groups identified within the age-related transcriptome changes. Interestingly, depression is not considered to be age-gated, as rates of diseases are consistently high throughout life (Kessler et al., 2003). This is in line with our prior (Erraji-Benchekroun et al., 2005) and current (Fig. S1) findings describing no correlative effects of depression on the trajectory of molecular aging. However, it is also important to note that many genes associated here with schizophrenia or bipolar disorder have also been implicated in major depression (e.g. BDNF, NPY, SST, HTR2AR, MAOB; Table 1). Here, the presence of subjects with diagnostics of depression in the investigated cohorts (see Supplemental information) may have had residual impact on the results, although we showed that the gene-expression correlates of depression are on average 200 times fewer than those of aging. Nevertheless it is a potential limitation of this study that will need to be addressed in larger studies of control subjects only. Additionally, the brain-area specificity of neurological disorders could potentially be partially accounted for by our data, as differences in rates of disease-gene-expression changes varied across areas by as much as 2.5 fold (Fig. 3). These results need to be repeated to confirm subtle differences between areas, because we cannot rule out the influence of chance variation. Additionally, as three of our tested brain areas were in frontal cortex, expansion of this study to additional disease-relevant brain areas such as hippocampus and striatum would be required to see if age-trajectories are steepest in the most disease affected areas. Finally, the specific anatomical localization of affected genes within the local microcircuitry (i.e. cortical layer or subcortical nuclei) may determine whether functional output may affect distal projections, as suggested in AD (Stam et al., 2007) or local microcircuitry, as suggested in SCZ (Lewis and Sweet, 2009), for instance.

In support of a genetic modulation or control of this molecular aging-by-disease risk model, we show that the cross-sectional trajectory of a large component of molecular aging was differentially affected in subjects carrying a common polymorphism in the SIRT5 putative longevity gene ($SIRT5_{prom2}$), which we also show correlated with reduced SIRT5 expression. Specifically, the greater age-down-regulation of mitochondrial-related subset of genes in association with $SIRT5_{prom2}$ suggests first that molecular aging may be affected in a modular fashion, and second that longevity genes may regulate patterns of transcript changes encompassing different disease pathways. Note here that the present investigation was supported by an *a priori* hypothesis for a putative SIRT5 effect, and that the molecular correlates of the $SIRT5_{prom2}$ variant were supported by FDR estimates. However, FDR takes into account a limited amount of information only, namely p-value, rank, number of significant transcripts, and total transcripts tested. It does not take into account other information present in the dataset such as multiple probesets for the same gene with convergent results, relatedness of genes found, and genes with convergent functional information. For example, we show that the gene set identified in correlation with $SIRT5_{prom2}$ has an estimated FDR of 19%, which can be interpreted as any single significant $SIRT5_{prom2}$-affected gene is estimated to have an 81% chance of being a true positive. However, the fact that 227 out of the 231 genes (98%) are in pro-aging directions suggests that the actual FDR may be lower. Additionally, congruent with the mitochondrial-localization of SIRT5 (Gan and Mucke, 2008; Nakagawa et al., 2009), many genes converge on a cellular compartment (mitochondria), code for different subunits of the same protein complex (Complex 1 for example; Fig. 5d), or are identified by significant effects on multiple probesets coding for the same gene present (Pink-1 and GTF3A for example), together increasing the confidence level in the biological validity of the findings, in addition to statistical criteria. Based on these results, we predict that SIRT5-risk allele (C/C) carriers may be at increased risk for mitochondrial dysfunction-related disorders, including Parkinson's and Huntington's diseases.

It is important to note, however, that this study was conducted using disease-free subjects and thus the observed losses of PINK-1 and DJ-1 are necessarily insufficient to cause PD. We conjecture that these changes may only be sufficient to cause disease in the context of other genetic and/or environmental risk factors and thus $SIRT5_{prom2}$ may only modulate age-of-onset of those who are otherwise predisposed to PD. Indeed, a study comparing array findings in striatum of PD brains to age-matched controls found decreases in many of the same genes we see affected by $SIRT5_{prom2}$, including multiple components of the electron transport chain, PINK-1 and DJ-1, but to a greater extent (1.2–8.6 fold decreases) in many cases than we observed (Simunovic et al., 2009). For example, they observed a ~2.2 and 8.6 fold decrease of PINK-1 and DJ-1 in striatum of PD subjects compared to age-matched controls (Simunovic et al., 2009), compared with our ~1.5 fold decreases seen in these genes at the oldest ages in $SIRT5_{prom2}$ risk-allele subjects. However, our subject ages only extended until age 71 in ACC, approximately the mean onset age of PD, and thus some of these subjects may have been on the trajectory to developing PD if they had lived to older ages. Alternatively, these subjects may have been protected from PD by other unidentified factors despite the potential increased risk conferred by the $SIRT5_{prom2}$ risk allele. Indeed, the $SIRT5_{prom2}$ risk allele cannot be a sole determinant of sporadic PD, as it is too prevalent in the population (~43–50%; Supplementary Table 9) compared with the prevalence of PD (1–3% in persons over 80; Tanner and Goldman, 1996). Thus, in line with the "common disease–common variant" hypothesis of disease, we speculate that $SIRT5_{prom2}$ may be one of many potential contributing factors for PD and/or other mitochondrial dysfunction-related diseases and declines.

This proof-of-concept study suggests that genetic modulation of molecular aging may associate with differential regulation of specific age-promoted disease pathways in the human brain. The confirmation of this model would have profound consequences for identifying

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genetic risk factors and for potential new drug development (i.e. SIRT5 targeting in Parkinson's). To this end, large-scale replication of these findings is needed in other post-mortem cohorts and in brain areas directly relevant to respective disorders (i.e. substantia nigra and basal ganglia for Parkinson's). Here, the association with reduced SIRT5 transcript levels suggests that either $SIRT5_{prom2}$ or closely-linked DNA variants may mediate the observed effects, but the extension of these findings to large-scale genetic studies combined with molecular aging assays will need larger test and replication cohorts. Further confirmation of the model will also necessarily come from assessment of live subjects with those neurological disorders and normal control cohorts to assess whether either disease onset and severity, or age-related functional declines (motor, cognition and emotionality for instance) are differentially associated with this particular SIRT5 snp or with other variants identified using the same methodological approach.

Additional limitations that will need to be addressed are the fact that changes were not assessed at the protein levels. Large-scale protein surveys are more limited in the number of peptides identified, and thus do not offer overviews of cellular functions as RNA surveys do. Nonetheless, while RNA levels may not correlate with protein levels in all cases, numerous studies corroborate our age-related RNA results at the protein or function levels (see brief review in Erraji-Benchekroun et al. (2005)). Here, we used transcriptome profiles to assess molecular brain aging as a whole (at least one "snapshot" of it). Mechanisms by which SIRT5 exerts an effect of molecular aging will need to be addressed at multiple levels of protein function (level, localization, function, etc.), which was beyond the aim of this study.

Concluding, our findings suggest a hypothesis for a uniting gene-expression level mechanism for age-of-onset across neurological diseases that is congruent with a "common disease–common variant" hypothesis. Confirmation of this model and investigation of the extent and specificity of the proposed brain age-related biosignature to peripheral transcriptomes may provide new avenues for predicting disease onset and trajectory, and potentially for designing novel therapeutic approaches through monitoring rates of molecular brain aging (by proxy in peripheral blood for example or by advancing neuroimaging techniques), in concert with assessment of novel age-related genetic risk factors (e.g. $SIRT5_{prom2}$) and associated biological (e.g. mitochondrial dysfunction) or phenotypic (e.g. cognitive decline) mediators.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.nbd.2010.09.016.

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