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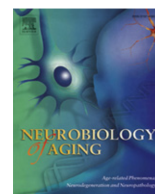

Age-associated changes in gene expression in human brain and isolated neurons

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Previous studies have suggested that there are genes whose expression levels are associated with chronological age. However, which genes show consistent age association across studies, and which are specific to a given organism or tissue remains unresolved. Here, we reassessed this question using 2 independently ascertained series of human brain samples from 2 anatomic regions, the frontal lobe of the cerebral cortex and cerebellum. Using microarrays to estimate gene expression, we found 60 associations between expression and chronological age that were statistically significant and were replicated in both series in at least 1 tissue. There were a greater number of significant associations in the frontal cortex compared with the cerebellum. We then repeated the analysis in a subset of samples using laser capture microdissection to isolate Purkinje neurons from the cerebellum. We were able to replicate 5 gene associations from either frontal cortex or cerebellum in the Purkinje cell dataset, suggesting that there is a subset of genes which have robust changes with aging. Of these, the most consistent and strongest association was with expression of *RHBDL3*, a rhomboid protease family member. We confirmed several hits using an independent technique (quantitative reverse transcriptase polymerase chain reaction) and in an independent published sample series that used a different array platform. We also interrogated larger patterns of age-related gene expression using weighted gene correlation network analysis. We found several modules that showed significant associations with chronological age and, of these, several that showed negative associations were enriched for genes encoding components of mitochondria. Overall, our results show that there is a distinct and reproducible gene signature for aging in the human brain.

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1. Introduction

Aging is a multidimensional phenomenon in which many aspects of function and phenotype in organisms change over time and appears to be a product of programmed (i.e., genetic) aspects, and stochastic events (Martin, 2011). The molecular mechanisms underpinning the biology of aging remain poorly defined. Identifying molecular events that vary along the lifespan has implications for understanding the causes of age-related phenotypes. For example, telomeres shorten with aging (Kirkwood, 2011; Shammas, 2011) and it has been suggested that telomere shortening plays a causal role in aging (Sahin et al., 2011). Robust molecular events that occur with aging might also act as markers of the aging process.

Previous studies of aging have examined gene expression using systems approaches, such as microarrays. There are several genes that show linear relationships between expression levels and chronological age in different tissues and species (Bahar et al., 2006; Colantuoni et al., 2011; Fraser et al., 2005; Kadish et al., 2009; Lu et al., 2004; Nakamura et al., 2012; Oh et al., 2011; Rodwell et al., 2004; Zahn et al., 2006, 2007). It has also been shown that there are biologically related groups of transcripts that robustly change with age. For example, mitochondrial gene expression is age responsive (Hamatani et al., 2004; Liu et al., 2011) and mitochondria might play a causal role in aging (Tranah, 2011).

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However, it is often not clear whether gene expression correlations with age are robust when comparing different tissues and organisms. One reason for this is that the magnitude of gene expression changes during aging in adults are relatively small compared with those seen, for example, during development (Colantuoni et al., 2011). It additionally appears that different tissues age at different rates and there might be species differences in gene expression in aging (Fraser et al., 2005; Miller et al., 2010; Oh et al., 2011; Zahn et al., 2007), which limits the ability to examine concordance across tissues and organisms. For example, a previous study suggested that there are more robust age-related changes in human cerebral cortex compared with the cerebellum and that the patterns of changes differed between humans and chimpanzees (Fraser et al., 2005). Other studies in human brain have identified differences in age-related gene expression in men and women (Berchtold et al., 2008), further demonstrating the complexity of identifying expression markers of aging.

In human tissues, genetic diversity also contributes to variation in gene expression (Gibbs et al., 2010). Furthermore, in some tissues cellular heterogeneity might also affect measurements of gene expression, especially if the cellular composition of tissue changes with aging. In the brain where there are many types of neurons and glia, age-dependent loss of neurons would lead to apparent associations with gene expression. Some previous studies have addressed this by examining general markers for neurons and glia (Lu et al., 2004) and others have used cell isolation techniques including laser capture microdissection (LCM) to clarify gene expression patterns in Parkinson's disease (Zheng et al., 2010) and in Alzheimer's disease (Blalock et al., 2011). However, it remains to be clarified to what extent any specific gene expression change with aging can be replicated in neurons, especially in the human brain.

Overall, these considerations suggest that though gene expression might be a marker of the aging process, it would be of interest to further examine age-associated expression in robust well-powered datasets. Our primary aim in the current study was to identify a single gene or group of genes that are correlated with aging, preferentially those that show a linear relationship with age. To identify such genes, we analyzed 2 large series of human brain samples (Hernandez et al., 2012; Trabzuni et al., 2011) for association with age and messenger RNA expression in the frontal cortex and cerebellum using a single microarray platform by rearraying previously collected samples. These brain areas were chosen because of previous data in smaller sample series (Fraser et al., 2005) suggesting differences in the number of age-related associations between the 2 regions. Additionally, to understand whether such changes can be detected specifically in neurons, we repeated the analyses in Purkinje cells isolated by LCM. Finally, we also examined gene networks associated with aging to test the hypothesis that networks might robustly detect changes in expression not seen at the level of single genes (Miller et al., 2008). Overall, our results support the hypothesis that there are reliable associations between gene expression and chronological age in the human brain.

2. Methods

2.1. Samples

We performed messenger RNA expression analyses from 2 human brain regions, the frontal region of the cerebral cortex and the cerebellum. Two independent series were obtained, a discovery set of 249 subjects from the University of Maryland (Gibbs et al., 2010; Hernandez et al., 2012) and a replication set from the Edinburgh Sudden Death Brain and Tissue Bank (Trabzuni et al., 2011). These brain samples have previously been examined by microarrays (Gibbs et al., 2010; Trabzuni et al., 2011), but on different platforms. Therefore, to provide a consistent dataset, we generated data using a single microarray platform (see below). After quality control, we used 203 samples in the discovery dataset and 73 in the replication dataset. Both sample series included male and female subjects with an age range of 15–91 years (mean, 35.2; median, 30) for the discovery set and a range of 16–83 years (mean, 50.4; median, 52.5) for the replication dataset. Demographic details of the cases are listed in Supplementary Table 1.

2.2. RNA extraction and processing

Frozen tissue samples of the posterior lobes of the cerebellar cortex, or the superior frontal cerebral cortex (Brodmann area 8 and 46) were obtained from neurologically normal Caucasian subjects. Aliquots of 100–200 mg of frozen tissue (predominantly gray matter) were carefully subdissected from each tissue for all subjects and used for expression assays. RNA was extracted using Trizol, biotinylated, and amplified using the Illumina TotalPrep-96 RNA Amplification Kit.

For LCM, we took frozen sections of cerebellum from a subset ($n = 98$; age range, 14–72) of samples from the discovery dataset. Tissue was immersed in Shandon M-1 embedding matrix (Thermo Electron Corporation, Rockford, IL, USA) and stored at −80 °C until use. Cryostat sections (7–8 μm thick) were cut and stained with Cresyl Violet (Ambion, Austin, TX, USA). LCM was performed with ArcturusXT microdissection system (Arcturus, Mountain View, CA, USA). Between 70 and 150 excised Purkinje cells were selected from the slide surface and captured on LCM Macro Caps. High-quality cellular RNA was recovered from the collected cells using PicoPure RNA isolation kit (Arcturus) and treated with RNase-free DNase (Qiagen, Valencia, CA, USA). The quality of RNA was analyzed using an Agilent 2100 bioanalyzer (Agilent, Foster City, CA, USA). For arrays, we used samples with an RNA integrity number (RIN) greater than 5.0 (range, 5–8.5) with 2 samples excluded because of low RIN. Two rounds of amplification were carried out with the Ambion MessageAmp II aRNA kit.

2.3. Microarrays

Amplified RNA from either bulk tissue extracts or LCM Purkinje cells were hybridized onto HumanHT-12_v3 Expression BeadChips (Illumina). These arrays contain 48,804 probes estimating expression of approximately 25,000 annotated genes from the RefSeq (Build 36.2, release 22) and Unigene (Build 199).

2.4. Correlations between gene expression and age

Each sample used in this series has been genotyped for common DNA variants as described elsewhere (Gibbs et al., 2010; Hernandez et al., 2012). We used genotype data to perform a principal components analysis for identity by state of genotype and took the first 2 principal components, PC1 and PC2, from this analysis to estimate overall genetic distance within the sample series. Cubic spline normalization was applied to raw output from array scans, then expression values were corrected for known covariates of sex, postmortem interval (PMI), principal components PC1 and PC2 from genotyping as above, and hybridization batch using multivariate regression as outlined previously (Gibbs et al., 2010) except that age was not specified in the model. The values for each of these parameters for each sample are listed in Supplementary Table 1.

The residuals for expression after covariate correction were then tested against age using linear regression. p values were adjusted for multiple testing using a false discovery rate (FDR) correction set at 0.05. Probes were included if detected in >95% of samples in each

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