

Gene expression profiling of aging in multiple mouse strains: identification of aging biomarkers and impact of dietary antioxidants

Sang-Kyu Park,^{1,*} Kyoungmi Kim,^{2,†} Grier P. Page,^{2,‡} David B. Allison,² Richard Weindruch³ and Tomas A. Prolla¹

¹Department of Genetics and Medical Genetics, University of Wisconsin, Madison, WI 53706, USA

²Department of Biostatistics, Section on Statistical Genetics and Clinical Nutrition Research Center, University of Alabama, Birmingham, AL 35294, USA

³Department of Medicine and Wisconsin Primate Research Center, Veterans Administration Hospital, University of Wisconsin, Madison, WI 53706, USA

Summary

We used DNA microarrays to identify panels of transcriptional markers of aging that are differentially expressed in young (5 month) and old (25 month) mice of multiple inbred strains (129sv, BALB/c, CBA, DBA, B6, C3H and B6C3F₁). In the heart, age-related changes of five genes were studied throughout the mouse lifespan: complement component 4, chemokine ligand 14, component of Sp100-rs, phenylalanine hydroxylase and src family associated phosphoprotein 2. A similar analysis in the brain (cerebellum) involved complement component 1q (alpha polypeptide), complement component 4, P lysozyme structural, glial fibrillary acidic protein and cathepsin S. Caloric restriction (CR) inhibited age-related expression of these genes in both tissues. Parametric analysis of gene set enrichment identified several biological processes that are induced with aging in multiple mouse strains. We also tested the ability of dietary antioxidants to oppose these transcriptional markers of aging. Lycopene, resveratrol, acetyl-L-carnitine and tempol were as effective as CR in the heart, and α -lipoic acid and coenzyme Q₁₀ were as effective as CR in the cerebellum. These findings suggest that transcriptional biomarkers of aging in mice can be

used to estimate the efficacy of aging interventions on a tissue-specific basis.

Key words: aging; antioxidants; biomarkers; caloric restriction; microarray; mouse.

Introduction

Gene expression profiling with DNA microarrays can measure the transcriptional changes of thousands of genes simultaneously providing a useful tool for the study of complex biological processes, such as aging. To understand the molecular basis of aging and to identify biomarkers of aging, we have used DNA microarrays to characterize tissue-specific gene expression profiles associated with aging in mice (Lee *et al.*, 1999, 2000, 2002; Higami *et al.*, 2004). Heart aging is characterized by a transcriptional profile suggestive of induction of cellular structural proteins involved in cardiomyocyte hypertrophy, a metabolic shift from fatty acid toward carbohydrate metabolism and reduced protein biosynthesis (Lee *et al.*, 2002; Park *et al.*, 2008). In contrast, brain aging in mice is associated with a heightened cellular immunity, inflammation and a concerted induction of genes involved in stress response (Lee *et al.*, 2000; Park *et al.*, 2008). In addition, the gene expression pattern is suggestive of reduced protein turnover and a decreased expression of genes encoding growth and trophic factors. Because these studies were performed in a very limited set of mouse inbred strains, it is unclear if the main observations represent general aging features, or if they are secondary to strain-specific pathology.

Transcriptional profiles of tissues from animals on caloric restriction (CR) suggest that CR reduces endogenous damage and induces metabolic shifts and thus opposes the aging process (Lee *et al.*, 1999, 2000, 2002; Park & Prolla, 2005a,b). Genome-wide microarray analysis of hepatic RNA shows that shifting from control diet to CR induces a rapid shift toward the gene expression profile of long-term CR and shifting from long-term CR to control diet reverses 90% of the CR effect on gene expression within 8 weeks, suggesting a cause- and effect relationship between the rate of aging and the CR-associated alterations in gene expression (Dhahbi *et al.*, 2004).

Several natural and synthetic compounds with antioxidant activity also have the potential to slow specific aspects of the aging process. In a previous study in the mouse heart, α -lipoic acid (LA) and coenzyme Q₁₀ (CQ) inhibited age-related alterations in the expression of genes involved in the extracellular matrix (ECM), cellular structure, and protein turnover, but had no impact on longevity or tumor patterns compared with control mice (Lee *et al.*, 2004). Resveratrol (RE), a polyphenol compound

Correspondence

Tomas A. Prolla, 5302B Genetics/Biotechnology building, 425 Henry Mall, Madison, WI 53706, USA. Tel.: (608) 265 5204; fax: (608) 262 2976; e-mail: taprola@wisc.edu

*Present addresses: Institute for Behavioral Genetics, University of Colorado at Boulder, 1480 30th St, Boulder, CO 80303, USA.

†Division of Biostatistics, Department of Public Health Sciences, UC Davis School of Medicine, Davis 95616, CA, USA.

‡Statistics and Epidemiology RTI International, Atlanta, GA 30341, USA.

Accepted for publication 12 May 2009

found in red wine, retards cardiac aging in mice (Barger *et al.*, 2008a,b) and increases survival of mice fed a high fat diet (Baur *et al.*, 2006). The yellow curry spice compound curcumin (CU) is more potent than vitamin E in scavenging free radicals (Zhao *et al.*, 1989) and reduces oxidative damage in an Alzheimer transgenic mouse model (Lim *et al.*, 2001), suggesting that it may also retard brain aging. Lycopene (LY), a major carotenoid present in tomato, reduces lipid peroxidation induced by oxidative stress (Parfitt *et al.*, 1994). Astaxanthin (AS) is a carotenoid responsible for the pink color of the flesh of salmon and also exhibits potent antioxidant properties in membranes (Palozza & Krinsky, 1992). Dietary supplementation with acetyl-L-carnitine (AC) in rats reverses the age-associated decline of mitochondrial functions (Hagen *et al.*, 1998a,b). Chronic treatment with the superoxide dismutase mimetic tempol (TP) shows protective effects on age-related vascular dysfunction in rats (Tatchum-Talom & Martin, 2004). Despite compelling evidence that these agents may retard specific aspects of aging, they have not been systematically or comparatively evaluated in their ability to modify aging parameters. Because lifespan studies are time consuming and costly, the ability to screen compounds for their ability to impact aging or mimic CR in specific tissues would be useful in deciding what compounds to pursue in further study, and also in deciding the most effective combinations of compounds.

In this study, age-related differential expression of genes from several strains of mice, 129sv, BALB/c, CBA, DBA, B6, C3H, and B6C3F₁, was determined using Affymetrix high-density oligonucleotide arrays to establish a panel of transcriptional markers of aging and to identify pathways significantly altered with aging in multiple mouse strains. We identified tissue-specific panels of biomarkers of aging that are common in multiple strains of mice in the heart and brain (cerebellum). Using these panels, we tested the effect of middle-age (15 month) onset CR and dietary supplementation of eight antioxidants (LA, CQ, RE, CU, LY, AC, AS and TP) on the expression of each transcriptional biomarker of aging.

Results

Identification of transcriptional biomarkers of heart aging

Comparison between 5-month-old (C5) and 25-month-old (C25) heart tissues with Affymetrix Mouse Genome 430A arrays representing 22 626 transcripts resulted in age-related changes ($P < 0.05$) of 3383 (15%) transcripts in the 129sv strain, 2552 (11%) in BALB/c, 1363 (6%) in CBA, 2449 (11%) in DBA, 2845 (13%) in B6, 1452 (6%) in C3H and 1718 (8%) in B6C3F₁. Among these, only 20 genes were common in at least six of the seven strains of mice tested (Table S1, Supporting information). From the binomial distribution, the probability of at least six of seven tests being significant at the 0.05 level by chance alone is 1.05×10^{-7} . Interestingly, 19 of the genes meeting these criteria were up-regulated with aging. There was only one down-regulated gene, enoyl coenzyme A hydratase 1, and its

Table 1 Age-related fold change of selected biomarkers of heart aging

Gene	Strain						
	129	BalbC	CBA	DBA	B6	C3H	B6/C3H
C4	2.1	2.1	2.1	3.7	1.9	2.0	1.8
Csprs	13.4	7.1	1.8	4.8	10.0	3.8	11.1
Pah	2.3	4.5	2.1	2.0	3.7	2.9	4.2
Cxcl14	2.0	2.8	1.6	2.0	1.5	1.7	2.4
Scap2	1.9	1.5	1.3	1.5	1.4	1.9	1.4

Values are the fold change (FC) of genes obtained by comparing the 25-month-old control group (C25) with the 5-month-old control group (C5). All genes are significantly ($P < 0.05$) changed in expression in all strains tested.

fold change (FC) with aging is relatively small in all strains (~ 0.85). Table 1 contains a panel of biomarkers of heart aging that were common in all seven strains of mice tested. Three genes are known to be involved in cellular immune and inflammatory responses, which is suggestive of heightened immunity in the aged heart. Complement component 4 (C4) is involved in the classical complement activation and chemokine (C-X-C motif) ligand 14 (Cxcl14) is a cytokine involved in immune responses (Shurin *et al.*, 2005). Src family associated phosphoprotein 2 (Scap2) is a specific substrate for the Src family protein tyrosine kinase Fyn (Marie-Cardine *et al.*, 1998) and is also known to negatively regulate cell proliferation. A recent study in Scap2^{-/-} mice suggests that Scap2 is required for proper activation of the immune system (Togni *et al.*, 2005). Phenylalanine hydroxylase (Pah) metabolizes aromatic amino acids and is involved in clearing circulating phenylalanine in blood and body fluids, increased levels of which can cause phenylketonuria (Christensen *et al.*, 2005). The biggest FC with aging was observed in component of Sp100-rs (Csprs) which encodes a putative G-protein coupled receptor (Weichenhan *et al.*, 2001). We note that the method used for identification of aging transcriptional markers is based on analysis of two time points, and therefore does not identify genes that are significantly altered only after 25 months of age, or genes that reach significance at earlier ages but at 25 months return to an expression level more similar to that of young animals.

Identification of transcriptional biomarkers of cerebellum aging

Of the 45 037 transcripts screened using Affymetrix Mouse Genome 430 2.0 arrays, 3752 (8%) transcripts were significantly changed by aging in the cerebellum of the 129sv strain, 4317 (10%) in BALB/c, 7273 (16%) in CBA, 4635 (10%) in B6, 9020 (20%) in C3H and 5948 (13%) in B6C3F₁. In the cerebellum, 99 genes were common in all strains tested: 82 of these were increased in expression and 12 were decreased in all strains tested (Table S2, Supporting information). Among them, we selected five genes having a higher FC with aging for further study (Table 2). Many genes involved in cellular immune and inflammatory responses were induced with aging. Four initiators

Table 2 Age-related fold change of selected biomarkers of cerebellum aging

Gene	Strain					
	129	BalbC	CBA	B6	C3H	B6/C3H
C4	1.9	1.8	3.9	3.3	3.5	2.3
C1qa	4.3	2.7	2.8	3.0	4.0	3.3
Ctss	2.6	1.8	2.7	2.3	2.2	2.7
Lzp-s	13.5	5.3	8.1	4.8	5.1	5.4
Gfap	3.6	2.6	3.2	2.3	4.0	2.6

Values are the fold change (FC) of genes obtained by comparing the 25-month-old control group (C25) with the 5-month-old control group (C5). All genes are significantly ($P < 0.05$) changed in expression in all strains tested.

of the classical complement cascade, C4 and three polypeptides of complement component 1q (C1q), were increased in expression by aging in all strains of mice, which supports a chronic inflammatory state of the aged cerebellum. Several cathepsins were also up-regulated in the aged cerebellum, including cathepsin D, S and Z. Cathepsin S (Ctss) plays a key role in major histocompatibility complex (MHC) class II-mediated antigen presentation (Hsieh *et al.*, 2002; Boes *et al.*, 2005). Lysozyme and P lysozyme structural (Lzp-s, also known as Lzp-1) are bacteriolytic lysosomal hydrolases, and involved in human hereditary amyloidosis (Röcken *et al.*, 2006). In all six strains of mice, Lzp-s showed the largest FC with aging. Glial fibrillary acidic protein (Gfap) was first discovered as an astrocyte-specific intermediate filament (Eng *et al.*, 1971) and is widely used as a marker of neurodegeneration (Nawashiro *et al.*, 2002; Wei *et al.*, 2002; Rozovsky *et al.*, 2005). In addition, two lipid molecule transporters, apolipoprotein D (ApoD) and E (ApoE), were induced with aging in the cerebellum. ApoD is also known to be involved in the response to oxidative stress in the brain (Navarro-Incio & Tolivia-Fernandez, 2004), and we have previously shown that its expression is increased with aging in the brain of mice, rhesus monkeys and humans (Loerch *et al.*, 2008).

The kinetics of expression of transcriptional biomarkers of aging and the effect of caloric restriction

We measured changes in mRNA levels for selected biomarkers of aging at various points of the mouse lifespan using real-time quantitative RT-PCR, and also tested the ability of CR to inhibit these changes. mRNA was extracted from the tissues of B6 mice ($n \geq 5$) killed at 5, 10, 15, 20, 25, 30 months of age and used as a template for real-time quantitative RT-PCR. In the heart, there was an age associated, approximate linear induction of expression in Csprs and Pah (Fig. 1). The expression of C4 was only slightly increased until 25 months of age and then greatly increased in 30-month-old animals. Both Cxcl14 and Scap2 showed a rising and falling expression pattern between 15 and 25 months and a significant induction at the age of 30 months. In the heart, CR appeared to prevent the late (25–30 months) age-related induction of these markers, with minimal effects

earlier in life. Measurement of heart function using echocardiogram analysis showed that there was no decline in heart function with aging until late age (25 months) in these strains (data not shown).

In the cerebellum, the expression of C1q alpha polypeptide (C1qa), Ctss and Gfap displayed an age-related linear increase throughout the lifespan of B6 mice (Fig. 2). The age-associated induction of C4 and Lzp-s was observed largely after 20 months of age. Interestingly, C4, a biomarker of aging common in both heart and cerebellum, followed the same expression pattern throughout the lifespan in both tissues, suggesting that this gene may be a very good biomarker of aging in postmitotic tissues. CR significantly opposed age-related transcriptional changes in the cerebellum (Fig. 2). CR showed a consistent effect on aging markers throughout the lifespan, including C1qa, Ctss and Gfap. CR reduced expression of Gfap and C1qa as early as 5 months of age (Fig. 2).

Identification of common pathways in heart and cerebellum aging of multiple mouse strains

To determine to what extent aging is associated with the alteration of shared pathways in multiple inbred mouse strains, we performed parametric analysis of gene set enrichment (PAGE), a computational method that allows determination of significant changes in defined gene sets (Kim & Volsky, 2005). For our analysis, we used GO biological pathway, cellular component and molecular function gene sets. We also calculated z ratios for each gene set, which serve as a normalization factor (Cheadle *et al.*, 2003). An initial comparative analysis between the seven mice strains revealed no gene sets significantly enriched ($P < 0.05$) among all strains in the heart. Relaxing the criteria to at least six strains significantly changed out of seven tested identified several enriched GO gene sets. A robust observation was the induction of genes involved in the complement and innate immune response, including complement activation (GO:0006956), acute inflammatory response (GO:0002526) and activation of plasma proteins during inflammation (GO:0002541) (Fig. 3). We had previously reported age-related induction of the complement system in the heart of B6C3H F1 hybrid mice (Lee *et al.*, 2002). Our study extends this finding to an additional six strains of inbred mice, and shows that complement activation is a universal feature of aging in the mouse heart. Gene sets involved in mRNA processing and splicing were also altered in expression, but the direction of change as determined by the z ratio varied between strains (Fig. 3). Interestingly, the GO term polysaccharide binding (GO:0030247) was significantly and consistently up-regulated in most strains. A gene significantly up-regulated in this gene set in some strains is Chitinase-3-Like 1 (CHI3L1). CHI3L1 is a secreted 40 kDa glycoprotein that is up-regulated in a number of human cancers and in non-neoplastic disease states characterized by chronic inflammation and tissue remodeling (Coffman, 2008). Stabilin 1, a receptor expressed on both macrophages and different sub-

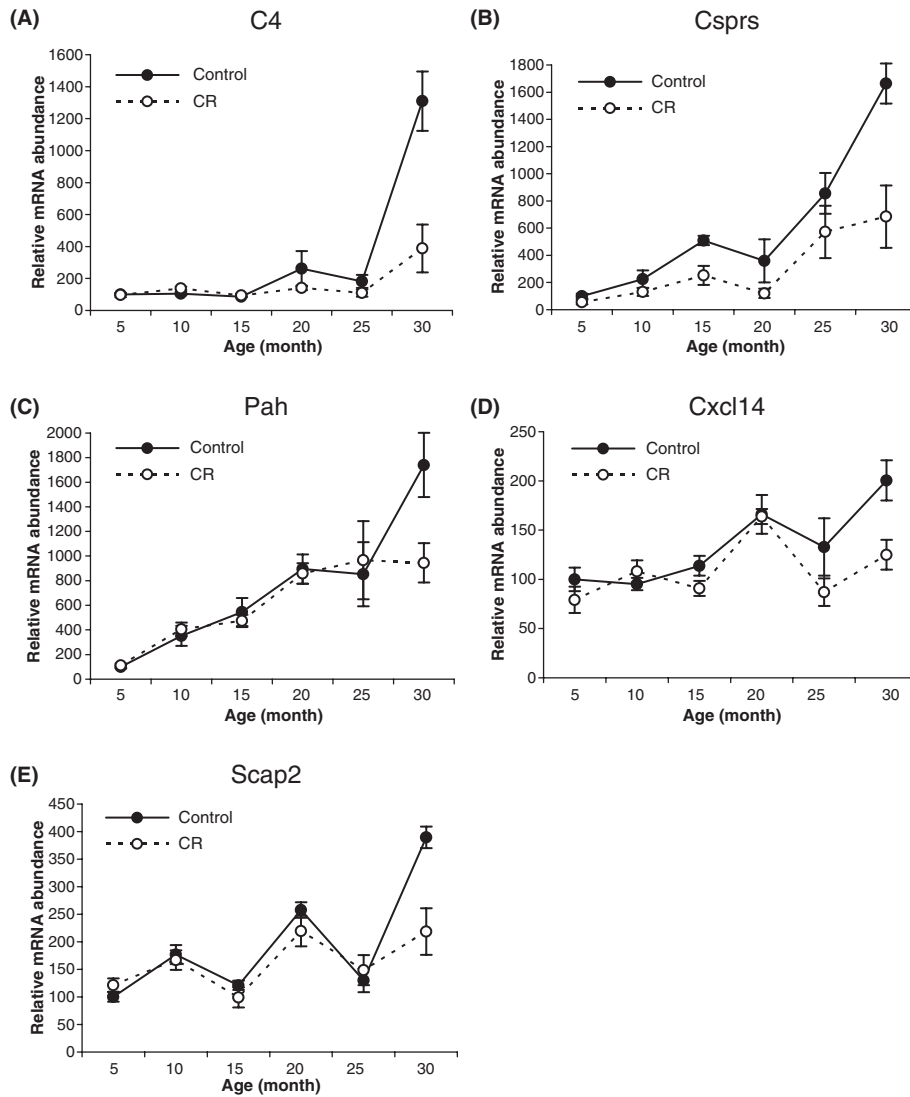


Fig. 1 Time-course quantitative RT-PCR of biomarkers of heart aging in B6 mice. The effect of aging and caloric restriction on the expression of selected biomarkers of heart aging is measured. Relative amount of mRNA compared to young control is shown for each sample. (A) C4, (B) Csprs, (C) Pah, (D) Cxcl14 and (E) Scap2. C5, 5-month-old control group. Lines above/below bars indicate standard error.

types of endothelial cells that is induced during chronic inflammation and tumorigenesis (Kzhyshkowska *et al.*, 2006), was also consistently induced. Several other members of this gene set are consistent with alterations in the ECM with aging in the heart.

A similar analysis in cerebellum revealed common gene sets altered in multiple mouse strains with aging. Inflammatory gene sets such as positive regulation of immune system process (GO:0002684) and regulation of immune response (GO:0050776) were induced with aging. A key feature of induction of these gene sets is also the activation of complement genes, including C4B (Rostagno *et al.*, 2002). Gene sets related to lysosomal activity, such as lysosome (GO:0005764) and lytic vacuole (GO:0000323) were also consistently induced across multiple mouse strains (Fig. 4). Gene set members changed in expression included several lysosomal proteases, such as cathepsins S, D, A and H. Cathepsins are involved in ECM

proteolytic degradation, and the induction of cathepsins in the brain is a marker of astrogliosis and microglial activation (Akahoshi *et al.*, 2007). Interestingly, the expression of cathepsin D appears to be a hallmark of aging in dogs and the human Alzheimer brain as well (Bi *et al.*, 2003). An imbalance of cathepsins, and defective lysosomes has been postulated to play an important role in human age-related neuronal dysfunction (Nakanishi, 2003). Many other GO terms were significantly changed in expression in at least five of six strains analyzed, but the direction of change as evidenced by the z-score, was not uniform. Surprisingly, gene sets related to mitochondria were strongly and significantly induced in some strains, but reduced in others (Fig. 4). Our overall analysis suggests induction of transcription of innate immunity genes in both tissues examined, as well as multiple tissue-specific patterns of aging.

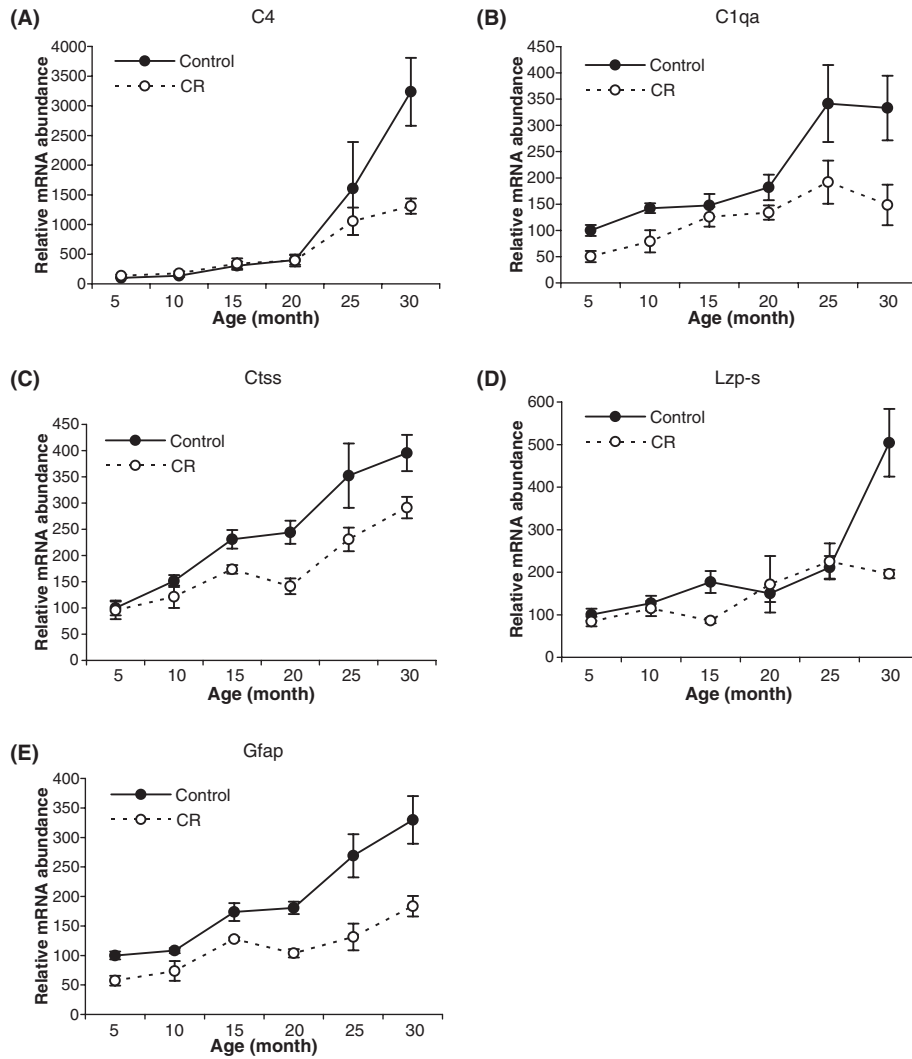


Fig. 2 Time-course quantitative RT-PCR of biomarkers of cerebellum aging in B6 mice. The effect of aging and caloric restriction on the expression of selected biomarkers of cerebellum aging is measured. Relative amount of mRNA compared to young control is shown for each sample. (A) C4, (B) C1qa, (C) Ctss, (D) Lzp-s and (E) Gfap. C5, 5-month-old control group. Lines above/below bars indicate standard error.

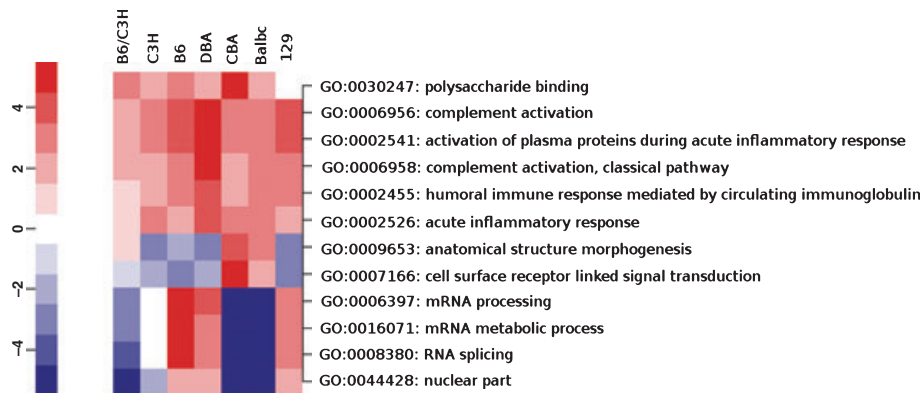


Fig. 3 Common pathways of heart aging in multiple mouse strains. Parametric analysis of gene set enrichment (PAGE) identified gene sets significantly enriched ($P < 0.05$) in heart aging among at least six strains of seven tested. Each row corresponds to the transcriptional alteration of each gene set with aging. Gene sets up-regulated with aging are shown in red, while gene sets down-regulated with aging are shown in blue. Labels indicate the GO numbers and GO term of each pathway.

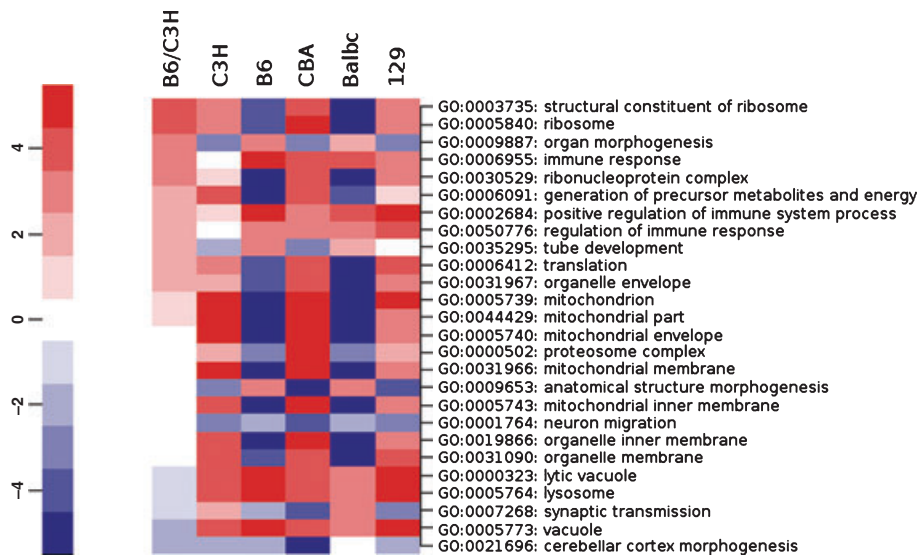


Fig. 4 Common pathways of cerebellum aging in multiple mouse strains. Parametric analysis of gene set enrichment (PAGE) identified gene sets significantly enriched ($P < 0.05$) in cerebellum aging among at least five strains of six tested. Each row corresponds to the transcriptional alteration of each gene set with aging. Gene sets up-regulated with aging are shown in red, whereas gene sets down-regulated with aging are shown in blue. Labels indicate the GO numbers and GO term of each pathway.

The effect of antioxidant supplementation on the expression of transcriptional biomarkers of aging

To determine the influence of selected antioxidants on the expression of biomarkers of aging in each tissue, 30-month-old mice fed various antioxidants from 15 months of age were compared with 30-month-old control fed mice (C30). As a positive control for aging retardation, we also analyzed 30-month-old mice on CR from 15 months of age to 30 months of age. We determined the effects of each intervention on the panel of aging markers, generating a tissue-specific 'aging prevention index' (API), representing the average effect of an intervention on all biomarkers. In the heart, middle age-onset CR significantly prevented age-related up-regulation of all six biomarkers of aging tested with an API value of 51 (Fig. 5). Among biomarkers of heart aging, only *Pah* was affected by all antioxidants tested. LA and CQ were not strongly effective in inhibiting age-related increases in expression of biomarkers of heart aging: only one gene, *Pah*, was affected by LA supplementation and two genes, *Pah* and *Scap2*, were affected by CQ (Table 3). There was a moderate anti-aging effect in CU- and AS-supplemented groups (API of CU and AS was 41% and 45%, respectively). Remarkably, AC and TP were even more effective than CR in terms of the API, and also significantly reduced the age-related expression of all biomarkers tested. RE, which we have previously shown to retard cardiac aging (Barger *et al.*, 2008a,b), and LY also showed strong efficacy in inhibiting the cardiac aging biomarkers.

The impact of middle age-onset CR and antioxidant supplementation on the expression of biomarkers of cerebellum aging is shown in Fig. 6 and Table 4. Similar to biomarkers of heart aging, all six biomarkers of cerebellum aging were markedly inhibited by CR (a 59% API). The age-associated up-regulation

of C1qa was decreased significantly by all eight antioxidant interventions (Fig. 6). As opposed to the effect on heart aging, LA and CQ were the two most effective antioxidants in suppressing age-related induction of biomarkers of aging in the cerebellum, nearly as effective as CR (API of LA and CQ were 58% and 50%, respectively). Supplementation of CU, LY and AC showed significant effects on the expression of five biomarkers of aging in the cerebellum. However, these antioxidants did not prevent the age-related up-regulation of C4 (Table 4). RE and TP, which were among the most effective antioxidants in heart aging, displayed only marginal efficacy in the cerebellum. These observations suggest that unlike the effect of CR, the effects of dietary antioxidant supplementation are tissue specific.

Discussion

In short-lived organisms, the examination of survival curves is practical, and therefore useful in assaying aging rates. However, for most mammalian species, survival curves are less practical due to their relatively long lifespan and complexity. Given the long lifespan of mammals, it would be useful to establish organ-specific biomarkers of aging for evaluating the efficacy of interventions. Our comparison of gene expression patterns in heart and cerebellum of multiple strains of mice revealed tissue-specific biomarkers of aging that can be used to measure the aging process. Although thousands of genes were changed in expression with aging in each individual strain, there was a relatively small number of common genes changed in all strains tested: 20 genes in the heart and 99 genes in the cerebellum. One possible explanation for this finding is that many biological processes associated with aging are strain specific. Aging resulted in up-regulation of several genes that are involved in immune and

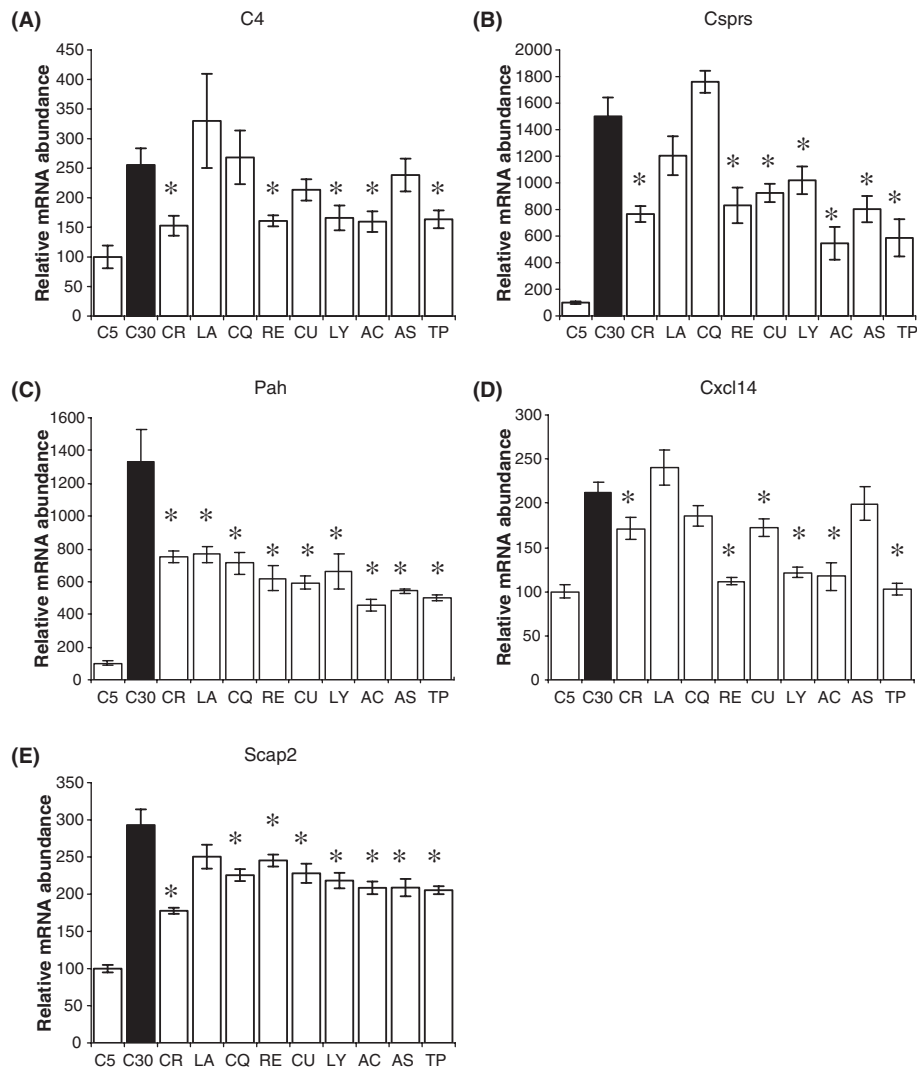


Fig. 5 The effect of middle age-onset CR and antioxidant supplementation on the expression of biomarkers of heart aging. Relative amount of mRNA compared to young control is shown for each group. (A) C4, (B) Csprs, (C) Pah, (D) Cxcl14 and (E) Scap2. C5, 5-month-old control; C30, 30-month-old control; CR, caloric restriction; LA, α -lipoic acid; CQ, coenzyme Q₁₀; RE, resveratrol; CU, curcumin; LY, lycopene; AC, acetyl-L-carnitine; AS, astaxanthin; TP, tempol. Lines above/below bars indicate standard error. The one-way ANOVA test showed significant *P*-value ($P < 0.01$) in all genes tested. *Significantly different from C30 ($P < 0.05$).

inflammatory responses related to innate immunity. The expression of C4 was induced with aging in both heart and cerebellum. C4 prevents early stage autoimmune disease (Paul *et al.*, 2002) and mice with a disrupted C4 locus showed an impaired immune response (Gadjeva *et al.*, 2002). In the heart, two more genes involved in the immune response were identified as biomarkers of aging. Cxcl14 is a potent chemoattractant that is ubiquitously expressed in normal tissues, but absent in many tumor cell lines (Frederick *et al.*, 2000) and Scap2 is required for the activation of the immune system (Togni *et al.*, 2005).

In addition to C4, several genes involved in the immune and inflammatory response were increased in expression with aging in the cerebellum, including the lysosomal proteases cathepsin D, cathepsin S, cathepsin Z, and three components of C1q (alpha, beta and gamma polypeptide) involved in innate

immunity. The lysosomal protease cathepsin S is involved in degradation of protein antigens and controls intracellular trafficking of class II MHC molecules (Hsieh *et al.*, 2002). The activation of the classical complement system was reported in the non-demented aged human brain and also in early-stage Alzheimer's disease (Zanjani *et al.*, 2005), and an increase of C1q beta polypeptide mRNA was found in aging rats (Pasinetti *et al.*, 1999). Taken together, our observations suggest that normal aging in the heart and brain is associated with a transcriptional pattern indicative of heightened immune and inflammatory responses. Interestingly, the expression of complement activation genes has been shown in skeletal muscle, kidney and brain in humans (Zahn *et al.*, 2006). The expression of innate immunity genes may be due to the activation of an ancient NF- κ B signaling pathway of host defense in multicellular organisms (Salminen *et al.*,

Table 3 Effect of CR or antioxidant supplementation on the expression of biomarkers of heart aging

Gene	CR	Antioxidant							
		LA	CQ	RE	CU	LY	AC	AS	TP
C4	66*	-48	-8	61*	27	58*	62*	11	59*
Csprs	52*	21	-19	48*	41*	34*	68*	50*	65*
Pah	47*	46*	50*	58*	60*	55*	71*	64*	67*
Cxcl14	37*	-24	24	90*	36*	81*	85*	12	98*
Scap2	60*	22	35*	25*	34*	39*	44*	44*	45*
API	51	16	19	52	41	51	66	45	66

The % inhibition effect of CR or antioxidants was computed as $[(O-S)/(O-Y)] \times 100$, where *O*, *S*, and *Y* are the average signal intensities of the 30-month-old control, 30-month-old CR or antioxidant-supplemented group, and 5-month-old control, respectively. API represents the average effect of the intervention on all markers tested.

CR, caloric restriction; LA, α -lipoic acid; CQ, coenzyme Q₁₀; RE, resveratrol; CU, curcumin; LY, lycopene; AC, acetyl-L-carnitine; AS, astaxanthin; TP, tempol.

*Significantly different between *S* and *O* groups ($P < 0.05$).

2008). This signaling system may connect genotoxic stress, inflammation, and apoptosis, and therefore play an important role in the origin of aging phenotypes and age-related diseases (Salminen *et al.*, 2008). Previous studies have shown that the expression of *Gfap*, the first validated brain aging transcriptional marker, increases progressively during aging in humans and rodent models (Nichols *et al.*, 1993). It is reassuring that our screen identified *Gfap*, and also that the expression of this gene is reduced by CR at all ages examined. Some of the inflammatory markers reported in this study were also identified in our original DNA microarray analysis of heart (Lee *et al.*, 2002) and brain (Lee *et al.*, 2000). Interestingly, gene sets related to the immune system, such as complement activation (GO:0006958) and regulation of the immune system (GO:0050776), were induced in both heart and cerebellum. Examination of these gene sets suggests that genes involved in innate immunity account for the majority of genes induced. Possibly, induction of these and other genes related to the immune system is a consequence of either increased levels of monocytes/macrophages in tissues, or increased levels of cytokines, as demonstrated in adipose (Wu *et al.*, 2007) and brain (Ye & Johnson, 1999) tissues of aged mice. In contrast, GO categories related to mitochondria were induced in some strains, but suppressed in others. This observation suggests that a reduction in the expression of genes related to mitochondria and energy metabolism is not a universal feature of aging in mice.

Our data revealed two biomarkers of aging that are common in both heart and cerebellum: C4 and tissue inhibitor of metalloproteinase 2 (TIMP2). Aging is a major risk factor for the development of arterial stiffness and vascular disease such as hypertension and atherosclerosis (Lakatta, 2002), and it is associated with the imbalance between matrix metalloproteinases and their endogenous inhibitors, tissue inhibitors of metalloproteinases (Dollery *et al.*, 1995; Zervoudaki *et al.*, 2003). In mice, new fibrovascular tissue from old animals expressed more TIMP2

than did corresponding tissue from young mice (Koike *et al.*, 2003). Comparison between young and old human microvascular endothelial cell lines revealed that TIMP2 is expressed at higher levels in cell lines from old humans (McNulty *et al.*, 2005). Interestingly, a transcriptional profiling of human tissues with aging identified TIMP1 as the gene displaying the highest change in gene expression in multiple tissues in humans (Zahn *et al.*, 2006). The transcriptional alterations of TIMP2 in multiple strains of aged mice are consistent with these previous observations, suggesting that elevated levels of TIMP2 may modulate impaired angiogenesis and fibrosis in aged tissues.

Previous studies reported the impact of antioxidants on age-related gene expression patterns in mice. Dietary supplementation with LA or CQ results in transcriptional changes associated with reduced oxidative stress in heart, but these antioxidants did not extend maximum lifespan and reduce tumor incidence (Lee *et al.*, 2004). Middle age-onset dietary supplementation of vitamin E also showed a partial inhibitory effect on age-related transcriptional alteration in heart and brain, but was not as effective as CR (Lee *et al.*, 2002; Park *et al.*, 2008). We have previously shown that RE can prevent age-related cardiac dysfunction and transcriptional alterations associated with cardiac aging (Barger *et al.*, 2008a,b), and these findings are in agreement with the strong effect of RE in inhibiting transcriptional markers of aging reported in this study. In the heart, AC was the natural compound that displayed the largest inhibition in the expression of the transcriptional markers. In rats, short-term supplementation with AC reduced age-related alterations in lipid metabolism in multiple tissues including normalization of the cholesterol/phospholipid ratio (Tanaka *et al.*, 2004) and reduced DNA damage in the brain (Haripriya *et al.*, 2005). We have previously identified transcriptional evidence for alterations in lipid metabolism as a major feature of aging in the heart, and showed that CR, but not dietary antioxidants, can prevent these alterations (Lee *et al.*, 2004; Park *et al.*, 2008). Thus, we postulate that similar to RE, AC may be acting to mimic the metabolic effects of CR in the heart.

We note that a major finding of this study is the remarkable difference in efficacy of the tested compounds. In the heart, RE, LY, AC, and TP were at least as effective as CR, whereas LA and CQ were the most effective antioxidants tested in the cerebellum. Our studies provide support for an important role of oxidative stress in aging, but suggest that the effects of individual antioxidants are tissue specific. Robust transcriptional biomarkers of aging will be useful for designing combinations of dietary antioxidants that will be effective in inhibiting the aging process in individual tissues in mammals.

Experimental procedures

Animals and dietary manipulations

Different strains of male mice were purchased from Harlan Sprague-Dawley at 6–7 weeks of age. Mice were housed singly in a pathogen-free facility and provided acidified water *ad libitum*.

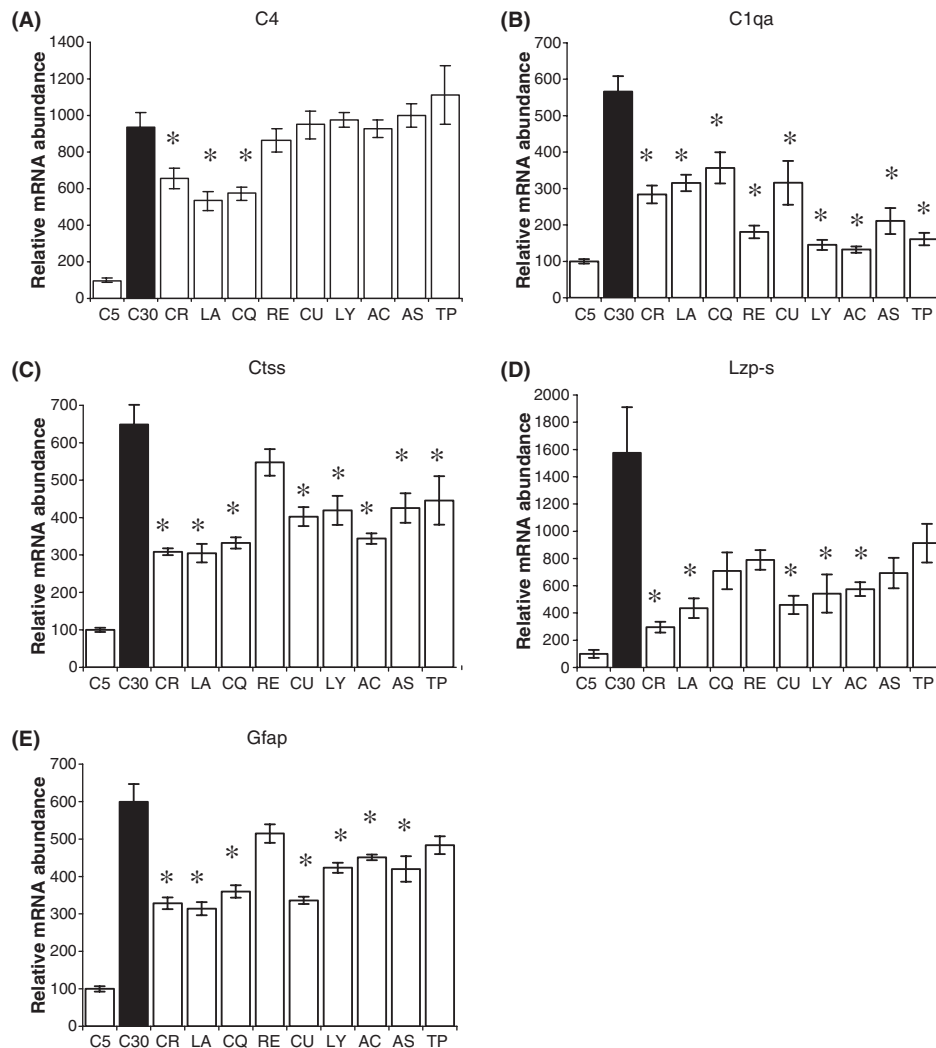


Fig. 6 The effect of middle age-onset CR and antioxidant supplementation on the expression of biomarkers of cerebellum aging. Relative amount of mRNA compared to young control is shown for each group. (A) C4, (B) C1qa, (C) Ctss, (D) Lzp-s and (E) Gfap. C5, 5-month-old control; C30, 30-month-old control; CR, caloric restriction; LA, α -lipoic acid; CQ, coenzyme Q₁₀; RE, resveratrol; CU, curcumin; LY, lycopene; AC, acetyl-L-carnitine; AS, astaxanthin; TP, tempol. Lines above/below bars indicate standard error. The one-way ANOVA test showed significant *P*-value ($P < 0.01$) in all genes tested. *Significantly different from C30 ($P < 0.05$).

Each mouse was fed 84 kcal per week of AIN-76A diet. For the study of CR, B6 mice were used: the control group was fed 84 kcal per week of AIN-76A and the CR group was fed 63 kcal per week (a 25% CR) from 5 months of age. To avoid malnutrition, the restricted diet was enriched in protein, vitamins, and minerals. The effect of dietary supplementation of antioxidant was performed in B6C3F₁ mice. Each group was fed 84 kcal per week of AIN-93M diet mixed with each antioxidant: LA (600 mg kg⁻¹ of diet), CQ (100 mg kg⁻¹ of diet), RE (50 mg kg⁻¹ of diet), CU (500 mg kg⁻¹ of diet), LY (250 mg kg⁻¹ of diet), AC (1 g kg⁻¹ of diet), AS (1 g kg⁻¹ of diet) and TP (5 mg kg⁻¹ of diet). The antioxidant groups were supplemented with each antioxidant since middle age, as we intended to test the effect of middle-age onset dietary supplementation. As a control, we also included mice under CR since 15 months of age. At the age of 30 months, mice were killed by

rapid cervical dislocation and tissues were immediately frozen in liquid nitrogen and stored at -80°C . All aspects of animal care were approved by the appropriate university committees and conformed to institutional guidelines.

RNA sample preparation and hybridization

Total RNA was extracted from frozen tissue and converted to double-stranded cDNA after purifying mRNA. Biotin-labeled cRNA was made from double-stranded cDNA and then hybridized to the gene chip as previously described (Lee *et al.*, 1999). Following hybridization, the gene chip was installed in a fluidics system for washes and staining. The signals on the gene chip were read using a Hewlett Packard GeneArray Scanner (Affymetrix, Santa Clara, CA, USA). The averaged images collected from two scanned images were used as raw data for statistical analy-

Table 4 Effect of CR or antioxidant supplementation on the expression of biomarkers of cerebellum aging

Gene	CR	Antioxidant							
		LA	CQ	RE	CU	LY	AC	AS	TP
C4	34*	48*	43*	8	-2	-5	0	-8	-21
C1qa	61*	54*	45*	83*	54*	90*	93*	76*	87*
Ctss	62*	63*	58*	18	45*	42*	56*	41*	37*
Lzp-s	87*	77*	59	53	76*	70*	68*	60	45
Gfap	54*	57*	48*	17	53*	35*	30*	36*	23
API	59	58	50	37	46	46	49	42	36

The % inhibition effect of CR or antioxidants was computed as $[(O-S)/(O-Y)] \times 100$, where *O*, *S*, and *Y* are the average signal intensities of the 30-month-old control, 30-month-old CR or antioxidant-supplemented group, and 5-month-old control, respectively. API represents the average effect of the intervention on all markers tested.

CR, caloric restriction; LA, α -lipoic acid; CQ, coenzyme Q₁₀; RE, resveratrol; CU, curcumin; LY, lycopene; AC, acetyl-L-carnitine; AS, astaxanthin; TP, tempol.

*Significantly different between *S* and *O* groups ($P < 0.05$).

sis. We used five animals per group, and hybridized each sample to independent DNA chips, because previous work from our laboratory suggests that variability between individuals is higher than variability observed in replicate hybridizations of the same samples (Weindruch et al., 2002).

Microarray data analysis

Preliminary data analysis was performed using Affymetrix algorithms for microarray data analysis, GCOS (GeneChip Operating Software). Detailed protocols for data analysis and extensive documentation of sensitivity and quantitative aspects of the method have been described previously (Lee et al., 2002). Gene expression change was called significant when the *P*-value (*P*) was < 0.05 . To obtain posterior true positive probabilities (pp) for each gene, we used a mixture modeling approach (Allison et al., 2002) that uses the frequentist *P*-values and incorporates them into a mixture model. The pp is the Bayesian probability that a gene is truly different between groups in mean expression level. The raw data of each DNA chip are provided as supporting information (Tables S3 and S4).

Pathway analysis of microarray data

To identify common pathways with aging in multiple inbred mouse strains, PAGE was employed (Kim & Volsky, 2005). We input a list of probe set IDs and their *t*-test statistic values to PAGE. For multiple test correction, we used Benjamini-Hochberg FDR estimate. Only GO terms that have at least ten and at most 1000 genes and have level 3 and below were analyzed. Probe set IDs filtered from the original series were used. The filtering process deletes the following probe set ids: probe sets ending with *x_at* and *s_at*, probe sets annotated with more than one gene, probe sets not mapped to a gene, and probe sets not with

the highest average SI for corresponding gene. Z-ratios were also determined for each gene set (Cheadle et al., 2003). All GO terms and pathways significantly altered with aging in each strain were shown in Tables S5 and S6, Supporting information.

Real-time quantitative RT-PCR

mRNA quantification was performed using real-time quantitative RT-PCR with ABI prism 7000 Sequence Detection System (TaqMan; Applied Biosystems, Foster City, CA, USA). Template mRNA was first converted to double-stranded cDNA and then amplified by *Taq* DNA polymerase. Gene-specific TaqMan probes contain a fluorescent reporter at the 5'-end of the probe. As the PCR cycle progresses, the degradation and release of the fluorescent reporter by *Taq* DNA polymerase results in fluorescence at 518 nm. The accumulation of PCR products, therefore, is detected directly by monitoring the increase in fluorescence during the amplification process. Gene-specific primers and probe sets were purchased from Assays-on-Demand Gene Expression probes (Applied Biosystems): Pah (Mm00500918_m1), C4 (Mm00437890_m1), Cxcl14 (Mm00444699_m1), Scap2 (Mm00490022_m1), C1qa (Mm00432142_m1), Lzp-s (Mm00657323_m1), Gfap (Mm00546086_m1), Ctss (Mm00457902_m1) and TATA binding protein (Tbp) (Mm00446973_m1). For Csprs and copine 2 (Cpne2), we designed our own primers and probe sets: for Csprs, 5'-GTT ATC CAT TGA ACT CTC CAT CCT TT-3' (forward primer), 5'-TGG TCC AAG TCC CAG CTA GAA-3' (reverse primer), and 5'-FAM-TGG ATT CTG CTA AGT ACA G-TAMRA-3' (fluorescent probe); for Cpne2, 5'-TCT CAG TGC TGT GTG TGC AAA G-3' (forward primer), 5'-TCC CGG TCC AGC AGG TT-3' (reverse primer) and 5'-FAM-CTG TCA GTG AGT GGC CA-TAMRA-3' (fluorescent probe). Tbp and Cpne2 were used as control genes for normalization.

Acknowledgments

We thank Roger Klopp for maintaining mouse strains and help with tissue collection. We also wish to thank members of the Prolla and Weindruch Labs for helpful advice and critical discussion. This work was supported by NIH grant RO1AG020681.

References

- Akahoshi N, Murashima YL, Himi T, Ishizaki Y, Ishii I (2007) Increased expression of the lysosomal protease cathepsin S in hippocampal microglia following kainate-induced seizures. *Neurosci. Lett.* **429**, 136–141.
- Allison DB, Gadbury GL, Heo M, Fernandez JR, Lee CK, Prolla TA, Weindruch R (2002) A mixture model approach for the analysis of microarray gene expression data. *Comput. Stat. Data Anal.* **39**, 1–20.
- Barger JL, Kayo T, Pugh TD, Prolla TA, Weindruch R (2008a) Short-term consumption of a resveratrol-containing nutraceutical mixture mimics gene expression of long-term caloric restriction in mouse heart. *Exp. Gerontol.* **43**, 859–866.

- Barger JL, Kayo T, Vann JM, Arias EB, Wang J, Hacker TA, Wang Y, Raederstorff D, Morrow JD, Leeuwenburgh C, Allison DB, Saupe KW, Cartee GD, Weindruch R, Prolla TA (2008b) A low dose of dietary resveratrol partially mimics caloric restriction and retards aging parameters in mice. *PLoS ONE* **3**, e2264.
- Baur JA, Pearson KJ, Price NL, Jamieson HA, Lerin C, Kalra A, Prabhu VV, Allard JS, Lopez-Lluch G, Lewis K, Pistell PJ, Poosala S, Becker KG, Boss O, Gwinn D, Wang M, Ramaswamy S, Fishbein KW, Spencer RG, Lakatta EG, Le Couteur D, Shaw RJ, Navas P, Puigserver P, Ingram DK, de Cabo R, Sinclair DA (2006) Resveratrol improves health and survival of mice on a high-calorie diet. *Nature* **444**, 337–342.
- Bi X, Head E, Cotman CW, Lynch G (2003) Spatial patterns of mammalian brain aging: distribution of cathepsin D-immunoreactive cell bodies and dystrophic dendrites in aging dogs resembles that in Alzheimer's disease. *J. Comp. Neurol.* **464**, 371–381.
- Boes M, van der Wel N, Peperzak V, Kim YM, Peters PJ, Ploegh H (2005) In vivo control of endosomal architecture by class II-associated invariant chain and cathepsin S. *Eur. J. Immunol.* **35**, 2552–2562.
- Cheadle C, Vawter MP, Freed WJ, Becker KG (2003) Analysis of microarray data using Z score transformation. *J. Mol. Diagn.* **5**, 73–81.
- Christensen R, Alhonen L, Wahlfors J, Jakobsen M, Jensen TG (2005) Characterization of transgenic mice with the expression of phenylalanine hydroxylase and GTP cyclohydrolase I in the skin. *Exp. Dermatol.* **14**, 535–542.
- Coffman FD (2008) Chitinase 3-like-1 (CHI3L1): a putative disease marker at the interface of proteomics and glycomics. *Crit. Rev. Clin. Lab. Sci.* **45**, 531–562.
- Dhabhi JM, Kim HJ, Mote PL, Beaver RJ, Spindler SR (2004) Temporal linkage between the phenotypic and genomic responses to caloric restriction. *Proc. Natl Acad. Sci. USA* **101**, 5524–5529.
- Dollery CM, McEwan JR, Henney AM (1995) Matrix metalloproteinases and cardiovascular disease. *Circ. Res.* **77**, 863–868.
- Eng LF, Vanderhaeghen JJ, Bignami A, Gerstl B (1971) An acidic protein isolated from fibrous astrocytes. *Brain Res.* **28**, 351–354.
- Frederick MJ, Henderson Y, Xu X, Deavers MT, Sahin AA, Wu H, Lewis DE, El-Naggar AK, Clayman GL (2000) In vivo expression of the novel CXC chemokine BRAK in normal and cancerous human tissue. *Am. J. Pathol.* **156**, 1937–1950.
- Gadjeva M, Verschoor A, Brockman MA, Jezak H, Shen LM, Knipe DM, Carroll MC (2002) Macrophage-derived complement component C4 can restore humoral immunity in C4-deficient mice. *J. Immunol.* **169**, 5489–5495.
- Gold B, Merriam JE, Zernant J, Hancox LS, Taiber AJ, Gehrs K, Cramer K, Neel J, Bergeron J, Barile GR, Smith RT; AMD Genetics Clinical Study Group, Hageman GS, Dean M, Allikmets R (2006) Variation in factor B (BF) and complement component 2 (C2) genes is associated with age-related macular degeneration. *Nat. Genet.* **38**, 458–462.
- Hagen TM, Ingersoll RT, Wehr CM, Lykkesfeldt J, Vinarsky V, Bartholomew JC, Song MH, Ames BN (1998a) Acetyl-L-carnitine fed to old rats partially restores mitochondrial function and ambulatory activity. *Proc. Natl Acad. Sci. USA* **95**, 9562–9566.
- Hagen TM, Wehr CM, Ames BN (1998b) Mitochondrial decay in aging. Reversal through supplementation of acetyl-L-carnitine and N-tert-butyl-alpha-phenyl-nitron. *Ann. N Y Acad. Sci.* **854**, 214–223.
- Haripriya D, Sangeetha P, Kanchana A, Balu M, Panneerselvam C (2005) Modulation of age-associated oxidative DNA damage in rat brain cerebral cortex, striatum and hippocampus by L-carnitine. *Exp. Gerontol.* **40**, 129–135.
- Higami Y, Pugh TD, Page GP, Allison DB, Prolla TA, Weindruch R (2004) Adipose tissue energy metabolism: altered gene expression profile of mice subjected to long-term caloric restriction. *FASEB J.* **18**, 415–417.
- Hsieh CS, deRoos P, Honey K, Beers C, Rudensky AY (2002) A role for cathepsin L and cathepsin S in peptide generation for MHC class II presentation. *J. Immunol.* **168**, 2618–2625.
- Kim SY, Volsky DJ (2005) PAGE: parametric analysis of gene set enrichment. *BMC Bioinformatics* **6**, 144.
- Koike T, Vernon RB, Gooden MD, Sadoun E, Reed MJ (2003) Inhibited angiogenesis in aging: a role for TIMP-2. *J. Gerontol. A Biol. Sci. Med. Sci.* **58**, B798–B805.
- Kzhyshkowska J, Gratchev A, Goerdts S (2006) Stabilin-1, a homeostatic scavenger receptor with multiple functions. *J. Cell Mol. Med.* **10**, 635–649.
- Lakatta EG (2002) Age-associated cardiovascular changes in health: impact on cardiovascular disease in older persons. *Heart Fail. Rev.* **7**, 29–49.
- Lee CK, Klopp RG, Weindruch R, Prolla TA (1999) Gene expression profile of aging and its retardation by caloric restriction. *Science* **285**, 1390–1393.
- Lee CK, Weindruch R, Prolla TA (2000) Gene-expression profile of the ageing brain in mice. *Nat. Genet.* **25**, 294–297.
- Lee CK, Allison DB, Brand J, Weindruch R, Prolla TA (2002) Transcriptional profiles associated with aging and middle age-onset caloric restriction in mouse hearts. *Proc. Natl Acad. Sci. USA* **99**, 14988–14993.
- Lee CK, Pugh TD, Klopp RG, Edwards J, Allison DB, Weindruch R, Prolla TA (2004) The impact of alpha-lipoic acid, coenzyme Q10 and caloric restriction on life span and gene expression patterns in mice. *Free Radic. Biol. Med.* **36**, 1043–1057.
- Leinase I, Holers VM, Thurman JM, Harhausen D, Schmidt OI, Pietzcker M, Taha ME, Rittirsch D, Huber-Lang M, Smith WR, Ward PA, Stahel PF (2006) Reduced neuronal cell death after experimental brain injury in mice lacking a functional alternative pathway of complement activation. *BMC Neurosci.* **7**, 55.
- Lim GP, Chu T, Yang F, Beech W, Frautschy SA, Cole GM (2001) The curry spice curcumin reduces oxidative damage and amyloid pathology in an Alzheimer transgenic mouse. *J. Neurosci.* **21**, 8370–8377.
- Loerch PM, Lu T, Dakin KA, Vann JM, Isaacs A, Geula C, Wang J, Pan Y, Gabuzda DH, Li C, Prolla TA, Yankner BA (2008) Evolution of the aging brain transcriptome and synaptic regulation. *PLoS ONE* **3**, e3329.
- Marie-Cardine A, Verhagen AM, Eckerskorn C, Schraven B (1998) SKAP-HOM, a novel adaptor protein homologous to the FYN-associated protein SKAP55. *FEBS Lett.* **435**, 55–60.
- McNulty M, Spiers P, McGovern E, Feely J (2005) Aging is associated with increased matrix metalloproteinase-2 activity in the human aorta. *Am. J. Hypertens.* **18**, 504–509.
- Nakanishi H (2003) Neuronal and microglial cathepsins in aging and age-related diseases. *Ageing Res. Rev.* **2**, 367–381.
- Navarro-Incio AM, Tolivia-Fernandez J (2004) The involvement of apolipoprotein D in pathologies affecting the nervous system. *Rev. Neurol.* **38**, 1166–1175.
- Nawashiro H, Huang S, Brenner M, Shima K, Hallenbeck JM (2002) ICP monitoring following bilateral carotid occlusion in GFAP-null mice. *Acta Neurochir. Suppl.* **81**, 269–270.
- Nichols NR, Day JR, Laping NJ, Johnson SA, Finch CE (1993) GFAP mRNA increases with age in rats and human brain. *Neurobiol. Aging* **14**, 421–429.
- Palozza P, Krinsky NI (1992) Astaxanthin and canthaxanthin are potent antioxidants in a membrane model. *Arch. Biochem. Biophys.* **297**, 291–295.

- Parfitt VJ, Rubba P, Bolton C, Marotta G, Hartog M, Mancini M (1994) A comparison of antioxidant status and free radical peroxidation of plasma lipoproteins in healthy young persons from Naples and Bristol. *Eur. Heart J.* **15**, 871–876.
- Park SK, Prolla TA (2005a) Gene expression profiling studies of aging in cardiac and skeletal muscles. *Cardiovasc. Res.* **66**, 205–212.
- Park SK, Prolla TA (2005b) Lessons learned from gene expression profile studies of aging and caloric restriction. *Ageing Res. Rev.* **4**, 55–65.
- Park SK, Page GP, Kim K, Allison DB, Meydani M, Weindruch R, Prolla TA (2008) alpha- and gamma-Tocopherol prevent age-related transcriptional alterations in the heart and brain of mice. *J. Nutr.* **138**, 1010–1018.
- Pasinetti GM, Hassler M, Stone D, Finch CE (1999) Glial gene expression during aging in rat striatum and in long-term responses to 6-OHDA lesions. *Synapse* **31**, 278–284.
- Paul E, Pozdnyakova OO, Mitchell E, Carroll MC (2002) Anti-DNA autoreactivity in C4-deficient mice. *Eur. J. Immunol.* **32**, 2672–2679.
- Röcken C, Becker K, Fändrich M, Schroeckh V, Stix B, Rath T, Kähne T, Dierkes J, Roessner A, Albert FW (2006) ALys amyloidosis caused by compound heterozygosity in exon 2 (Thr70Asn) and exon 4 (Trp112Arg) of the lysozyme gene. *Hum. Mutat.* **27**, 119–120.
- Rostagno A, Revesz T, Lashley T, Tomidokoro Y, Magnotti L, Braendgaard H, Plant G, Bojsen-Møller M, Holton J, Frangione B, Ghiso J (2002) Complement activation in chromosome 13 dementias. Similarities with Alzheimer's disease. *J. Biol. Chem.* **277**, 49782–29790.
- Rozovsky I, Wei M, Morgan TE, Finch CE (2005) Reversible age impairments in neurite outgrowth by manipulations of astrocytic GFAP. *Neurobiol. Aging* **26**, 705–715.
- Salminen A, Huuskonen J, Ojala J, Kauppinen A, Kaarniranta K, Suuronen T (2008) Activation of innate immunity system during aging: NF-kB signaling is the molecular culprit of inflamm-aging. *Ageing Res. Rev.* **7**, 83–105.
- Shurin GV, Ferris R, Tourkova IL, Perez L, Lokshin A, Balkir L, Collins B, Chatta GS, Shurin MR (2005) Loss of new chemokine CXCL14 in tumor tissue is associated with low infiltration by dendritic cells (DC), while restoration of human CXCL14 expression in tumor cells causes attraction of DC both in vitro and in vivo. *J. Immunol.* **174**, 5490–5498.
- Tanaka Y, Sasaki R, Fukui F, Waki H, Kawabata T, Okazaki M, Hasegawa K, Ando S (2004) Acetyl-L-carnitine supplementation restores decreased tissue carnitine levels and impaired lipid metabolism in aged rats. *J. Lipid Res.* **45**, 729–735.
- Tatchum-Talom R, Martin MS (2004) Tempol improves vascular function in the mesenteric vascular bed of senescent rats. *Can. J. Physiol. Pharmacol.* **82**, 200–207.
- Togni M, Swanson KD, Reimann S, Kliche S, Pearce AC, Simeoni L, Reinhold D, Wienands J, Neel BG, Schraven B, Gerber A (2005) Regulation of in vitro and in vivo immune functions by the cytosolic adaptor protein SKAP-HOM. *Mol. Cell. Biol.* **25**, 8052–8063.
- Wei LC, Shi M, Chen LW, Cao R, Zhang P, Chan YS (2002) Nestin-containing cells express glial fibrillary acidic protein in the proliferative regions of central nervous system of postnatal developing and adult mice. *Brain Res. Dev. Brain Res.* **139**, 9–17.
- Weichenhan D, Kunze B, Winking H, van Geel M, Osoegawa K, de Jong PJ, Traut W (2001) Source and component genes of a 6-200 Mb gene cluster in the house mouse. *Mamm. Genome* **12**, 590–594.
- Weindruch R, Kayo T, Lee CK, Prolla TA (2002) Gene expression profiling of aging using DNA microarrays. *Mech. Ageing Dev.* **123**, 177–193.
- Wu D, Ren Z, Pae M, Guo W, Cui X, Merrill AH, Meydani SN (2007) Aging up-regulates expression of inflammatory mediators in mouse adipose tissue. *J. Immunol.* **179**, 4829–4839.
- Ye SM, Johnson RW (1999) Increased interleukin-6 expression by microglia from brain of aged mice. *J. Neuroimmunol.* **93**, 139–148.
- Zahn JM, Sonu R, Vogel H, Crane E, Mazan-Mamczarz K, Rabkin R, Davis RW, Becker KG, Owen AB, Kim SK (2006) Transcriptional profiling of aging in human muscle reveals a common aging signature. *PLoS Genet.* **2**, e115.
- Zanjani H, Finch CE, Kemper C, Atkinson J, McKeel D, Morris JC, Price JL (2005) Complement activation in very early Alzheimer disease. *Alzheimer Dis. Assoc. Disord.* **19**, 55–66.
- Zervoudaki A, Economou E, Stefanadis C, Pitsavos C, Tsioufis K, Aggeli C, Vasiliadou K, Toutouza M, Toutouzas P (2003) Plasma levels of active extracellular matrix metalloproteinases 2 and 9 in patients with essential hypertension before and after antihypertensive treatment. *J. Hum. Hypertens.* **17**, 119–124.
- Zhao BL, Li XJ, He RG, Cheng SJ, Xin WJ (1989) Scavenging effect of extracts of green tea and natural antioxidants on active oxygen radicals. *Cell Biophys.* **14**, 175–185.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

- Table S1** Age-related fold change of biomarkers of heart aging.
- Table S2** Age-related fold change of biomarkers of cerebellum aging.
- Table S3** Normalized signal intensities and absent/present calls of microarray data of heart.
- Table S4** Normalized signal intensities and absent/present calls of microarray data of cerebellum.
- Table S5** Pathways significantly altered with heart aging.
- Table S6** Pathway significantly altered with cerebellum aging.

As a service to our authors and readers, this journal provides supporting information supplied by the authors. Such materials are peer-reviewed and may be re-organized for online delivery, but are not copy-edited or typeset. Technical support issues arising from supporting information (other than missing files) should be addressed to the authors.