Review TRENDS in Neurosciences Vol.27 No.10 October 2004

Caliban's heritance and the genetics of neuronal aging

Bruce Teter[1] and Caleb E. Finch[2]

[1]Department of Medicine, University of California Los Angeles, 16111 Plummer Street, Sepulveda, CA 91343, USA
[2]Ethel Percy Andrus Gerontology Center and Department of Biological Sciences, University of Southern California, 3715 McClintock Avenue, Los Angeles, CA 90089-0191 USA

Although current research on brain aging is dominated by Alzheimer's disease (AD), many other brain changes arise during middle age in humans and in rodent models that are independent of AD-like neurodegeneration. Differences and continuities between normal and pathological aspects of neuronal aging reveal the relative contributions and interactions of genetic and environmental factors. Apolipoprotein E alleles might be prototypes for genetic polymorphisms associated with functional changes that arise during middle age. Mice are valuable models for these aspects of aging because most genotypes show little neurodegeneration, and none accumulate β-amyloid unless human transgenes are introduced. As further human genes are found to modify normal and pathological neuronal aging, this zoo of aging-animal variants will facilitate analysis both of pathways of age-related neuronal dysfunction and of environmental influences on these pathways.

'[Caliban], a born devil, on whose nature nurture can never stick…' – referring to Caliban, who was sired by a devil in Shakespeare's *The Tempest*.

Caliban's heritance, the genetic basis for neuronal aging, might have descended from ancient lineages but has only recently become widely shared. Since 1800, life expectancy has doubled, and advanced old age, once a rare stage in human life history, is becoming increasingly common. Thus, an increasing proportion of adults experience slowly progressing neurological deficits and degenerative changes after age 40, which was the life expectancy of premodern populations. Three major questions are considered. First, which gene systems underlie the canonical pattern of normal brain aging in humans and other mammals? Second, which gene systems modulate environmental influences on brain aging and neurodegenerative diseases? Third, how do genetic polymorphisms influence brain functions at different stages of life? In discussing these complex issues, this review will examine distinctions and continuities between normal and pathological aspects of neuronal aging, especially in relation to neuronal loss and early neuronal changes that manifest by middle age. Brain aging is also considered in relation to the evolution of life spans and the role of antagonistic pleiotropy – that is, processes that are adaptive earlier in reproductive life but that have adverse consequences in later stages of life.

Corresponding author: Caleb E. Finch (cefinch@usc.edu).

Paradigm shift: brain aging is not only neuron loss

The phenomena of neuronal aging have been discussed for more than 100 years in terms of three classical traits: neuron loss, neuron atrophy and aging pigment (lipofuscins). The hippocampus has received great attention because of its role in memory and its vulnerability to neuron loss from Alzheimer's disease (AD) and stroke. Neuron loss in most fields of the aging hippocampus is negligible in the absence of AD or vascular lesions, up through the eighth decade (human hilar neurons are a possible exception to this, although equivalent neurons in the rat show more robust stability [1]). However, neuron death during aging can occur in other brain regions in the absence of specific neurological diseases.

Although normal aging can occur without overt neuron loss, subcytocidal, yet progressive, atrophic changes are observed with increasing age. Numerous biochemical and structural changes that compromise neuron function are expressed as various neurodegenerative phenotypes and precede cell death and more gross changes. Neuron atrophy, characterized by dendritic and perikaryal atrophy and reduced levels of neurotransmitters and receptors, is widely observed during aging [2,3]. For example, levels of dopamine receptors are markedly decreased in normal humans before the age of 50; similar changes occur in monkey and rodent models [4,5]. The reversibility of atrophy by growth factors [2,6,7] and the attenuation of neurotransmitter receptor loss by caloric restriction [2] suggest that different processes occur in normal aging and the apoptotic or necrotic cell death observed during AD or vascular dementia. Concurrent with neuronal atrophic changes during midlife, glial changes begin, including white matter degeneration and hyperactivity of astrocytes and microglia; these complex interactions are outside the scope of this brief review [2,3,8].

The role of aging pigments in brain aging is still not entirely clear. Even large lipofuscin deposits do not always cause neuron death – for example, neurons of the inferior olive of elderly control brains accumulate enough lipofuscins to displace the cell nucleus but do not exhibit neuron loss [9]. Provisionally, the neuronal lipofuscins linked to normal aging appear different to the earlier-onset

 0166-2236/$ - see front matter doi:10.1016/j.tins.2004.08.005

neuronal ceroid lipofuscinoses that are associated with cell death.

Together, the cellular profile of brain aging (neuronal atrophy and receptor loss, increased deposits of lipofuscins, and glial hyperactivity) is remarkably consistent across mammals and defines a canonical pattern of aging [2,8]. The paradigm of aging has clearly shifted, from the received wisdom of global, inevitable neuronal cell death during aging to early manifestations of sublethal, yet functionally degenerative, changes. When is the onset of these changes and why does aging (simply time) promote their expression? Does time represent accumulations of cellular wear and/or environmental stresses, or are there intrinsic, genetic changes, acting alone or in concert with these time-dependent accumulations? Because of the stability of phenotypic aging traits across generations, strong genetic influences on neuronal aging are likely.

Caliban's heritance: the genetics of brain aging

How rigidly does aging act on the phenotypic expression of genes related to neurodegeneration? An age-dependent phenotype caused by one or more inherited genes can be apparent not only through fully penetrant changes in gene expression (as in familial AD) but also through genetic risk factors that interact with environmental effects [as with apolipoprotein E (ApoE) alleles]. The 'heritability' of complex traits gives a global scale of transmission of phenotypes across generations. Many examples show the important influence on brain and behavior of environmental interactions with genetic effects [10]. Shakespeare placed Caliban at one extreme of heritability, as one who resisted all environmental influence. Changes in heritability with age imply an alteration in the balance of environmental and genetic influences. The heritability of various forms of memory is fairly strong, with increasing genetic influence from childhood to adulthood, and possibly into old age [11]. Twin studies show strong heritable influences on cognition in aging, but also sporadic effects that could arise from developmental variations [8,11]. A major issue in aging is how the influence of a genotype during brain development can modify the vulnerability of adult neurons to environmental influences.

The genetics of brain aging might be more complex than in other tissues because, consistent with its cellular complexity, the brain expresses more genes than any other organ. Genetic polymorphisms and their variant phenotypes add to this complexity, particularly in heterozygotes. However, examples of well-known genetic polymorphisms (in the usual sense of population genetics) that associated with normal aging are now emerging from this complexity. ApoE4, the most general genetic risk factor for AD and cognitive decline, is a possible prototype for genetic polymorphism effects involving an extended prodromal stage that can not only worsen normal aging but also promote pathological neuronal aging. Curiously, many genes associated with AD and related disorders are also expressed in other tissues [e.g. ApoE, amyloid precursor protein (APP), presenilins, tau, insulin-degrading enzyme, tumor necrosis factor α (TNFα) and β-secretase], suggesting that aging changes in the brain could share mechanisms with aging changes in other tissues [12]. Other genes involved in AD are being investigated in several chromosomal regions that contain quantitative trait loci (QTLs) linked to AD, at 6p21, 10q24 and 11q23 [12]. This diversity of genes implies diverse preclinical etiological paths, and thus many opportunities for pharmacogenomic intervention in aging that might also apply to AD. Because aging is the only consistent risk factor in AD, research into the mechanisms of these genes in AD could reveal vulnerable points in age-related neurodegeneration that might apply to normal aging and its milder cognitive effects.

Amyloid plaques and neurofibrillary tangles

AD is pathologically defined by brain-region-specific accumulations of amyloid plaques composed of fibrillar β-amyloid (Aβ) protein and neurofibrillary tangles (NFTs) over threshold criteria; however, some accumulation of brain amyloid and NFTs often occurs by later ages in normal aging. So how do plaque and tangle loads interact with neuron atrophy, neuron death, and cognitive decline? Amyloid load is a notoriously poor correlate of AD cognitive deficits, whereas NFTs more strongly correlate with the degree of cognitive deficits in AD. Scattered NFTs occur in the parahippocampal cortex by middle age, yet little or no amyloid is usually present (Braak stages I and II) [13]. However, some cases lack NFTs, but have diffuse amyloid deposits [14].

Although associations of neuron death with excess Aβ remain strong, the neurocidal focus of research into Aβ has shifted from fibrillar (plaque-producing) to oligomeric (soluble) aggregates [15,16]. Oligomeric Aβ forms can cause sublethal aspects of neuronal and synaptic degeneration, as seen in transgenic mice overexpressing human APP isoforms, in which behavioral deficits occur well before plaque formation [15,17]. Consistent with early behavioral deficits, APP transgenic mice show deficits in long-term potentiation and synaptophysin, before amyloid plaques develop [18,19]. Remarkably, all APP transgenic models have little or no neuron loss in association with amyloid plaques [20]; this could be consistent with the notion that plaques are secondary effects of the disease or its interactions with aging. Furthermore, transgenic mice overexpressing human tau show extensive neuron loss in association with NFTs [21]. Thus, Aβ overproduction might cause early, pre-plaque cognitive deficits, whereas NFTs, following amyloid plaque formation, could be a factor in neuron loss later in AD. These early manifestations of sublethal neurodegeneration could lead to preclinical deficits in the absence of the extensive neuron loss that is characteristic of end-stage disease.

ApoE alleles as modulators of normal and pathologic neuronal aging

ApoE alleles have robust effects on brain functions during aging and neurodegeneration. ApoE3, the most common, is considered the benign allele. ApoE4 is a risk factor for AD (and shorter life span), accounting for $\sim$50% of non-familial AD cases in the USA. Depending on the population, ApoE4 increases AD risk 15–20-fold and accelerates onset of AD by 5–15 years [22]. At the cellular

level, ApoE4 alleles reduce neuronal sprouting with clear allele dose effects in AD [23] and in rodent models [24]. Most effects of ApoE4 are strongly influenced by allele dose and increased ApoE4 expression, consistent with the E4 phenotype as a gain of (negative) function [25]. However, occasional E4/E4 carriers survive to 90 years old with normal cognition [26]. ApoE4 can negatively influence other neurodegenerative disorders, with allele dose effects on amyotrophic lateral sclerosis, multiple sclerosis, traumatic brain injury and Parkinson's disease.

ApoE alleles also influence specific neuronal functions at later ages, in the absence of dementia. In healthy elderly brains, ApoE3 carriers, compared with ApoE4 carriers, have 20% more dendritic spines in hippocampal dentate granule neurons [27] (Figure 1) and a larger Golgi apparatus in cholinergic neurons [28]; these differences imply negative effects of ApoE4 on neuronal metabolism. Lacking information on ApoE carriers early in life, it is not known whether these differences were present from early life or developed later. Moreover, ApoE4 allele dose effects influence amyloid accumulation and oxidative damage [22], which could be major influences on neuronal function during normal aging. It would be interesting to reanalyze the perikaryal atrophy in cholinergic neurons [2] according to ApoE genotype. ApoE2, although the least common allele, is protective for AD; its association with longevity in centenarians [29] might be due cardiovascular benefits [30]. An open question is how ApoE alleles influence nutritional modulations of brain aging. Caloric restriction attenuates some neuron receptor loss during aging, as well as many brain glial and inflammatory changes [31].

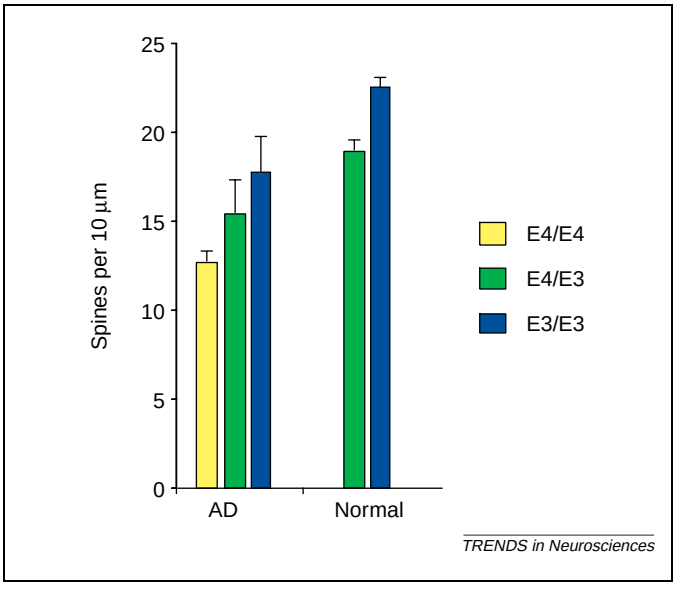

Figure 1. Apolipoprotein E (ApoE) alleles modulate normal and pathological neuronal aging beginning in midlife. In healthy, non-demented elderly brains, ApoE4 carriers (E4/E3) have 16% fewer dendritic spines in hippocampal dentate granule neurons than ApoE3 homozygotes (E3/E3). In Alzheimer's disease (AD), the effect of ApoE4 shows an allele-dose response: E4/E4 carriers display a 28% reduction in spine density [27]. This effect might originate in midlife because middle-aged ApoE4 transgenic mice have fewer dendritic spines than weanlings [27]. These early effects of ApoE4 indicate extended prodromal stages in AD and possibly general age-related cognitive changes. Using data from Ref. [27].

Early manifestations of ApoE effects

How early in life does ApoE4 affect brain structure and function? Recent sensitive measures have pushed back the earliest ages at which ApoE4 shows effects by measuring 'endophenotypes' identified by phenotypically homogeneous subpopulations [32]. Cognitive effects of ApoE4 are not seen on the general intelligence 'g-factor' in children [33,34]. The Nun Study [35] shows the earliest indication of cognitive deficits: ApoE4 carriers at age 22 had lower 'idea density' in essays, which predicted AD by age 80. This indication of early AD predisposition matches the Braaks' suggestion that prodromal AD stages are associated with sporadic NFTs in those in their twenties [36].

With increasing adult age, ApoE4 is associated with cognitive deficits and pathologic changes that foreshadow characteristics of AD. Of particular interest, ApoE4 is associated with lower brain glucose metabolism in non-demented, clinically normal individuals as young as in their thirties, in regions that are similarly affected in AD (the parietal, temporal and prefrontal cortex) [37,38]. Moreover, ApoE4 is associated with visuospatial attention deficits in individuals in their forties [39] and reduced hippocampal volume in those in their fifties [40]. These allelic effects might correspond to the observation of fewer dendritic spines in middle-aged ApoE4 transgenic mice than in weanlings [27]. These early effects of ApoE4 indicate extended prodromal stages in AD and possibly general age-related cognitive changes. The relationship between mild cognitive impairment (MCI) and later AD is not well defined. Although ApoE4 might have a general impact on cognition, this might reflect an enriched sample of preclinical AD patients [41–43]. The MCI phenotype can be combined with the ApoE4 genotype as a strong predictor (~95%) of imminent AD [44,45]. However, not all individuals with MCI go on to develop AD [26,46]. The relationship between MCI and AD was recently debated at the 2004 International Conference on AD and Related Disorders (http://worldeventsforum.com/alz/program2004.htm).

Genetic models of ApoE in brain aging and neurodegeneration

ApoE is expressed to different degrees in glia and neurons: it is secreted by astrocytes, whereas its presence in neurons could be largely due to ApoE uptake. Transgenic mice expressing human ApoE under the control of the human ApoE promoter have been developed as models for the human pattern of cell-specific expression of ApoE [47]. To evaluate the separate effects of ApoE expression in different cell types, transgenic mice have been developed that express human ApoE alleles in neurons [under control of the neuron-specific enolase (NSE) promoter] and astrocytes [under control of the glial fibrillary acidic protein (GFAP) promoter]. A commonly used transgenic host is the ApoE-knockout mouse, in which the lack of blood ApoE causes hypercholesterolemia [48], which itself might promote atypical neurodegenerative aging changes in these mice, as well as the NSE- and GFAP-promoter-driven transgenic mice [49]. Fortunately, the susceptibility to hypercholesterolemia is less in the human ApoE

promoter-driven transgenic mice and in newer transgenics with targeted replacement (TR) of the mouse ApoE gene by human ApoE isotypes [50]. ApoE4-TR mice have greater inflammatory responses to lipopolysaccharide (e.g. increased interleukin-6 and TNFα levels in brain and blood) than do ApoE3-TR mice, similar to what is observed in human carriers [50]; it is therefore unclear whether ApoE allele effects on neuronal aging are, in part, secondary to glial inflammatory processes.

In general, ApoE4 transgenic mice show greater cognitive deficits than ApoE3 transgenics. GFAP-ApoE4 mice show cognitive deficits without detectable AD-like pathology [51], whereas NSE-ApoE4 mice show age-related cognitive deficits in females only [52]. The defects observed in NSE-ApoE4 mice could be mediated by the toxicity of ApoE4 proteolytic fragments [53], as seen in AD brains.

ApoE transgenics also show allelic effects on AD-related pathologies. Phosphorylated tau accumulates more during aging in NSE-ApoE4 transgenic mice than in ApoE3 transgenic mice [53]. Importantly, the GFAP-ApoE transgenics did not show this effect on phosphorylated tau, implying that neuron-specific ApoE expression is associated with ApoE isoform-specific effects. In another transgenic model, the NSE-ApoE transgene has been combined with a familial AD APP transgene that elevates soluble Aβ at early ages and causes plaque formation later. In this model, when compared with the ApoE4 transgenic and ApoE-knockout mice, ApoE3 slowed synaptic loss in middle age prior to the appearance of Aβ deposits [54]. Complex effects of ApoE on Aβ accumulation in transgenic models support the hypothesis that plaque development is delayed most by ApoE2, to an intermediate extent by ApoE3 and least by ApoE4, as in AD [55].

Evolution of ApoE and aging

Neuronal aging might have evolved in relation to human-specific ApoE alleles. Chimpanzees and other great apes show very little evidence of neurodegeneration at their most advanced ages, in contrast to macaques, which develop many indications of AD-like neuropathology [56]. Yet the great apes have the same Aβ peptide as most other vertebrates (rodents are an exception, with substitutions that reduce aggregation) [56]. The remarkable conservation of the Aβ sequence in vertebrates implies an important role for it in neuronal and other functions.

ApoE is a candidate gene for the evolutionary modification of neuron aging. Great apes have an ApoE protein which is predicted to function like human ApoE3 in lipid-binding selectivity [56]. Some time in the past six million years, the great ape ApoE mutated to ApoE4, which is considered ancestral in the genus *Homo*. The unique human ApoE3 and ApoE2 are recently derived: ApoE3 is estimated to have occurred in humans ~200 000 years ago [57]. ApoE3 might have spread in response to selective pressures of a changing diet, including progressively more animal cholesterol and fats, which appear to be AD risk factors in some modern populations. The benefit of ApoE3 for health during middle age might have been a factor in the unique care-giving evolved by humans. The ApoE4 allele might persist in human populations by its support of greater inflammatory responses, which could be adaptive for acute injuries [50] and milder chronic effects of certain pathogens [58] (e.g. liver damage from the hepatitis C virus [59]). The effects of ApoE4 allele dose on blood cholesterolemia, although modest [56], imply that there might be diet(ApoE allele interactions in brain aging outcomes. Both the Aβ peptide and ApoE4 could be examples of antagonistic pleiotropy, with benefits at younger ages but maladaptive effects at later ages. Although expressed predominantly by glia, ApoE is associated with direct and indirect effects on neuronal aging. The ApoE polymorphisms illustrate how a continuum between normal and pathologic neuronal aging can be shifted by an evolutionarily recent allelic variant.

Other animal models

No single animal model could possibly represent the complexities of human aging, because of our vastly longer life span and more complex environmental exposure. Laboratory mice and rats do not accumulate brain amyloids during aging unless genetically modified. From this, the canonical mammalian aging pattern of neuronal atrophy and glial hyperactivity seems not to depend on prior accumulations of brain amyloids. Inbred rodents offer opportunities for genetic analysis because of wide differences in organ-specific diseases of aging and life span. For example, atrophy of cholinergic neurons is more prominent in aging Sprague-Dawley rats than in Brown Norway rats [2]. BALB/c and C57BL/6 mice also differ in aging of cholinergic and monoaminergic functions [60]. Moreover, the emerging genome maps of mouse strains could provide a wealth of gene targets for brain aging. The ongoing study of the four-way cross of common strains of mice (BALB/cJ, C57BL/6J, C3H/HeJ and DBA/2J) is yielding QTLs for life span and for aging of immune and other non-neural systems [61]. It would be cogent to identify QTLs for aging in brain and behavior in these mice and to consider correspondence to QTLs associated with AD in humans [12].

Two inbred mouse models of aging merit mention: senescence-accelerated mice (SAM) and dwarf mice. SAM strains have short life spans of 10–15 months in association with peripheral (but not brain) amyloidosis due to mutant ApoAII, a plasma protein [62]. Senescence-resistant (SAMR) strains live six months longer than senescence prone (SAMP) strains (although this is still shorter than most strains). SAMP10 mice are a model for cerebral degeneration and, by 10 months, show gross cholinergic neuron atrophy with loss of synaptophysin and postsynaptic density protein (PSD)-95 [63,64] and declining hippocampal APP expression [65]. SAMP8 mice show cholinergic neuron atrophy and learning impairments [63], and extracellular granules and vacuolation resembling scrapie infections [66,67].

Dwarf mice are one of the strangest new models for aging. Three dwarf mice that are deficient in pituitary growth hormone, growth hormone receptors or insulin-like growth factor live 3–4 years, which is up to 65% longer than conventional strains [68,69]. Among indications of slowed aging in these dwarf mice is the slower decline in locomotor activity and maze learning [68].

Future genetic factors for AD and longevity can be evaluated for direct effects in transgenic mice and invertebrate models. For example, transgenic fruitflies expressing mutant tau show many features of neurodegeneration, including neurofibrillary pathology; these flies are being used to identify genetic modifiers of tau neurotoxicity and new therapeutic targets [70].

Concluding remarks

The extended life expectancy and advanced old age of modern humans is revealing slowly progressing neurological deficits and degenerative changes that occur after the age of 40. The paradigm of aging has clearly shifted from inevitable neuronal cell death during aging to early manifestations of sublethal but functionally degenerative changes. These early age changes reveal both distinctions and continuities between normal and pathological aspects of neuronal aging. Strong genetic influences on neuronal aging are implicated by the stability of phenotypic traits across generations. Specific gene systems underlying the canonical pattern of normal brain aging in humans have come to light, particularly genetic polymorphisms that are associated with neuronal aging. ApoE4 might be a prototype for gene polymorphism effects in both normal and pathological neuronal aging. For example, ApoE4 reduces the number of neuronal dendritic spines in aged humans in the absence of dementia and in middle-aged transgenic mice. ApoE4 displays antagonistic pleiotropy and evolutionarily recent allelic variants (E2 and E3) shift its affected continuum between normal and pathologic neuronal aging. Many animal models that offer opportunities for genetic analysis of aging have been, and continue to be, developed.

Caliban's heritance is very real for brain aging at the species level. However, inherited traits of aging can be, at least somewhat, 'unstuck' from nature by nurture, which now includes diet, drugs and other interventions. As the genetic investigation of AD progresses, many more genes are likely to become candidates for modulating brain aging, using diverse cellular pathways and providing new targets for therapeutic intervention. The boundaries between 'usual' aging and definable neurological disease might become less clear as lifestyles and interventions enabling 'successful aging' further extend human life expectancy.

Acknowledgements

Caleb E Finch is a founder of Acumen Pharmaceuticals, Inc. and has potential conflicts in discussing soluble forms of Aβ in mechanisms of neurodegeneration; he is also supported by grants from The Alzheimer's Association, the John Douglas French Alzheimer's Foundation, and the NIA (AG 15940 and AG 18478). Bruce Teter is supported by a grant from NIA (AG 00962). We thank Greg Cole for critical helpful comments and Tom Wisniewski for providing the data for Figure 1 [27].

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