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CONSTITUTIONAL GENETIC MARKERS OF AGING

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Abstract — Constitutional genetic markers of aging can be defined as members of that subset of genes that modulate the times of onset and/or the rates of progression of one or more of the processes of aging, or the response of the target cells, tissues and organisms to a particular process. These genetic factors are classified into: (1) those that control changes in structure and function that may be universally expressed in aging organisms or that are expressed in large taxonomic groups of organisms ("public markers") and (2) those that control changes that are species specific or that reflect polymorphisms or mutations within a species ("private markers"). Both spontaneous and experimentally induced genetic variation can identify and characterize such genetic elements. Recommendations for implementing such a program of research include (1) *particularization* of the aging phenotype, (2) further development of nonmammalian models amenable to genetic analysis, (3) systematic search for relevant spontaneous mutations in *Mus musculus*, (4) utilization of recombinant inbred, chimeric, transgenic and interspecific mice and (5) investigations of genetic concomitants of speciation.

Key Words: genetics and aging, biomarkers, polymorphisms, mutations, progeroid syndromes, *Homo sapiens*, *Caenorhabditis elegans*, *Mus musculus*, speciation

INTRODUCTION

ONE CAN think about genetic markers of aging in two quite different ways — phylogenetically and ontogenetically. The latter would embrace the more traditional roles of biomarkers of aging, in which one assesses the postmaturational rates of accumulation of various changes in structure and function. A geneticist would focus upon changes in the primary structure of DNA, as well as upon nonmutational changes in the expression of genetic information. Such changes can be deterministic ("programmed") or stochastic (random, accidental events). Examples are given in the article on molecular markers of aging by Robert Shmookler Reis (p. 271 this issue). My major task, however, is to consider what I shall call constitutional genetic markers of aging. These can be defined as the subset of genes that modulate one or more of the processes of aging. Such modulation could influence the time of initiation (or termination) of a particular process, the rate of progression of a particular process, or the response of the target cells, tissues and organism to the process. Unfortunately, there is no definitive proof supporting any given process as a fundamental mechanism of aging. Genetic analysis, therefore, has to rely upon a variety of phenotypic properties which are believed to accompany biological aging. Before considering genetic influences, it is important to evaluate the conceptual rationale for deciding what phenotypes to consider.

The "Casarett rules"

Casarett (1964) proposed a set of guidelines to decide whether or not ionizing radiation can be shown to cause premature aging. These have since been extrapolated as guidelines for deciding the potential role of other agents, including certain constitutional genetic defects like the Werner syndrome (Epstein *et al.*, 1966). Support for the Casarett rules was reinforced by Epstein (1985), who came to the conclusion that the gene responsible for the Werner syndrome cannot be regarded as influencing the fundamental aging process, since the Casarett rules were not obeyed in that condition. These rules are as follows:

1. The agent "causes the force of mortality to increase earlier in the treated than in the nontreated control group, without alteration of the shape of the entire mortality curve."
2. The agent "brings forward proportionately in time the age of onset and the time of development of all the diseases or causes of death affecting the control group, without alteration of degree, sequence or absolute incidence of the disease and causes of death, and without induction of disease."
3. The agent "causes all the morphological and physiological manifestations of the aging process to appear and develop at proportionately earlier chronological ages, to degrees and rates in the various organs proportional to those degrees and rates in organs of nonirradiated animals."

Casarett correctly pointed out that the above formulations "may be oversimplified, more or less in error, and insufficiently quantified for correctly evaluating data . . ." The clear implication of the Casarett rules is that *a single agent* — be it a single gene or a single physical or chemical treatment — can faithfully reproduce all the phenotypic manifestations of aging. This would require that there exists only a single fundamental process of aging. A priori, these conclusions would seem exceedingly unlikely. Gerontologists are in general agreement that there are *multiple* processes of aging (Warner *et al.*, 1987). Even Casarett concluded his 1964 paper with the words "aging processes"!

Particularization of the aging phenotype

If we believe that no single agent can influence *all* of the manifestations of aging, then it is essential, in carrying out a genetic analysis, to specify the specific phenotype or phenotypes of interest. The global phenotype of maximum life span has obvious validity and is feasible for certain of the investigative approaches described below. It is clearly less feasible when applied to the study of human aging, however. Thus, one needs to consider a variety of changes in structure and function that are correlated with chronological aging but that may or may not be related to intrinsic biological aging.

Species specificity of ontogenetic markers

One traditional gerontological approach to deciding if a given marker is related to intrinsic aging, as opposed to chronological aging, is to compare the times of appearance or rates of accumulation of the markers among species having contrasting maximum life span potentials (MLSP). For the case of intrinsic aging, these parameters should be inversely related to MLSP. For the case of chronological aging, they should

TABLE 1. A CLASSIFICATION OF CONSTITUTIONAL FACTORS THAT MAY INFLUENCE VARIOUS ASPECTS OF AGING (SEE TEXT)

I. Genes controlling public markers
A. Universal genetic factors
B. Group-specific genetic factors
II. Genes controlling private markers
A. Species-specific genetic factors
B. Intraspecific genetic factors
1. polymorphisms
2. mutations

be strictly related to chronologic time, with essentially no differences among the species with contrasting MLSP. A more careful consideration of this issue, however, reveals some logical pitfalls. It is clearly the case that all manifestations of aging, qualitatively and quantitatively, cannot be identical in all species. Thus, the loss of proliferative homeostasis that characterizes aging in mammalian species (Martin, 1979) cannot possibly obtain in *C. elegans* or *D. melanogaster,* since, with the exception of the gonads, all of the cells of the adults of those species are postreplicative. While one could restrict such studies to groups of more closely related species, *a priori,* it may still be the case that, because of their genetic constitution, particular aspects of aging may be anomalous in one or more of these species. Moreover, even within a given species, unusual nature/nuture interactions among individuals may lead to considerable qualitative and quantitative variations in the playing out of aging in individual subjects.

Classification of constitutional genetic factors influencing aging

The above considerations suggest that it may be useful to outline a tentative classification of constitutional genetic factors that have the potential to influence various aspects of aging (Table 1).

Despite the concerns raised in the previous section, it is conceivable that there exists a *subset* of genes that play an important role in the aging of all or virtually all living multicellular organisms that exhibit senescence. We might refer to these as universal genetic factors. An example might be a gene or genes that control the rates of accumulation of lipofuscin pigments in aerobic organisms; although it may be the case that the precise mixture of types of these "aging pigments" vary from organism to organism, they have been observed in an astounding array of organisms (Table 2) (Martin, 1985).

A second logical category of genetic factors influencing aging would involve subsets of genes that have comparable effects with taxonomic groups, such as orders or families. A possible example of group-specific genetic factors has already been alluded to in connection with the comments on the loss of proliferative homeostasis in mammals. The fact that mice characteristically begin to develop monoclonal proliferations of cells, sometimes leading to a variety of cancers around 2 years of age, while humans do so at around 50 or 60 years of age, strongly implicates a subset of genes that modulate these processes. Among these could be genes that determine the stability of chromosomes in mitotic somatic cells, since a variety of chromosomal mutations have been shown to accompany aging (Martin *et al.*, 1985b) and to be characteristic of one or another stages

TABLE 2. EXAMPLES OF ORGANISMS IN WHICH THERE IS EVIDENCE OF AGE-RELATED ACCUMULATIONS OF LIPOFUSCIN PIGMENTS (AFTER MARTIN, 1985, WHICH SHOULD BE CONSULTED FOR REFERENCES)

Species name	*Common name*
Neurospora crassa	bread mold
Podospora anserina	dung fungus
Paramecium aurelia	paramecium
Caenorhabditis elegans	round worm
Bulla gouldiana	snail
Drosophila melanogaster	fruit fly
Musca domestica	house fly
Psittacula kameri	parrot
Mus domesticus	house mouse
Rattus norvegius	laboratory rat
Cavia porcellus	guinea pig
Felus catus	domestic cat
Canis familiaris	domestic dog
Sus scrofa	pig
Macaca mulatta	rhesus monkey
Homo sapiens	human

in the natural history of most, if not all, neoplasms (Sandberg and Wake, 1981; Yunis, 1986).

Putative universal and group-specific genetic factors can be regarded as those controlling public markers of aging, in that virtually all organisms (for the case of universal factors) and virtually all organisms within a taxonomic grouping (for the case of group-specific factors) would be subject to such genetic controls.

I would like to suggest, however, that we gerontologists give consideration to the validity of genetic factors that control what might be referred to as private markers of aging (Table 1) (Martin, 1987). One category might be those that are unique to or especially characteristic of a particular species of organisms. An example in *Homo sapiens* might be the apparently unique propensity of aging humans to develop masses of paired helical filaments (neurofibrillary tangles) in subsets of central nervous system neurons. These are characteristic of the pathology of dementia of the Alzheimer type (Mann and Sinclair, 1978), a very prevalent disease of the aged. These lesions are noted, to a lesser degree, in elderly individuals without a clinical or neuropathological diagnosis of Alzheimer's disease (Dayan, 1970; Morimatsu *et al.*, 1975). Although not specific for Alzheimer's disease in human subjects, paired helical filaments are not characteristic markers of aging in other mammals, nor can they be experimentally induced in other mammalian species.

A second category of genetic factors that control private markers are those that are responsible for *intraspecific* genetic variation. They in turn can be classified as either polymorphisms or mutations. By convention, polymorphisms are defined as Mendelian or monogenetic traits that can be found in a given population in at least two forms, neither of which occurs with a frequency of less than 1 to 2% (Vogel and Motulsky, 1987). In view of the comparatively high frequencies of the minor alleles in such polymorphisms, they are generally regarded as normal variants, but homozygotes, heterozy-

gotes or double heterozygotes for the minor alleles might, in some instances, be especially vulnerable to some age-related alterations in structure and function. I cannot give an ideal example, but one possible illustration might be the genetic variation at the locus that codes for alpha 1-antitrypsin, a glycoprotein that inhibits neutrophil elastase, a proteolytic enzyme capable of degrading multiple components of connective tissues. That gene is highly polymorphic, more than 30 different haplotypes having been described (Gadek and Crystal, 1982; Fagerhal and Cox, 1981). Most individuals carry alleles of the M family, the combined frequencies of the haplotypes of these being about 0.90 among caucasians (Dykes *et al.*, 1984). The S and Z haplotypes have frequencies, respectively, of around 0.02–0.04 and 0.01–0.04 and 0.01–0.02 (reviewed by Nukiwa *et al.*, 1986). MZ heterozygotes and, more convincingly, SZ double heterozygotes, are prone to develop chronic obstructive pulmonary disease as they age (Geddes *et al.*, 1977; Fagerhal and Cox, 1981; reviewed by Nukiwa *et al.*, 1986). Might such a disorder then become, for this small, but significant portion of the population, a private marker of aging, or should it be simply regarded as an age-related disease? Most gerontologists would probably respond that it should be regarded as a special disease, but I urge that we give the alternative view some serious consideration. For the affected individuals, that particular dysfunction is a reasonably predictable outcome of aging. For those individuals, there are very important clinical considerations as to the prevention and management of age-related disabilities. Moreover, if, via a founder effect, the allele frequencies were drastically changed and there were little or no selective pressures against the new major allele (quite unlikely for the case of our example of S and Z alleles, but quite possible for the case of alleles that had phenotypic expression substantially after the cessation of reproductive function), the loss of pulmonary function would come to be regarded as the "normal" or "usual" way people age and die.

Point mutations, of course, occur at much lower frequencies (approximately 10^{-4} to 10^{-9}) within all populations of organisms (Dobzhansky, 1951), although the concept of "hot spots" (genetic loci that exhibit unusually high frequencies of mutation) is well established (Benzer, 1955).

Assume that, because of such autosomal dominant mutation, a post-reproductive individual who was normal at birth, who developed normally and who reproduced normally exhibits a novel pattern of aging. Other members of the pedigree exhibit similar unusual patterns of aging. Is this a disease or can we say mutant individuals are displaying private markers of aging?

To cite a concrete example, consider the well-established autosomal dominant known variously as Azorean Neurologic Disease, Joseph Disease or Machado–Joseph disease (McKusick, 1986). It has been observed in a large number of descendants of one William Machado, a native of an island in the Portuguese Azores. A progressive ataxic gait is generally the first manifestation, but this is not usually apparent until after the age of 40. Although there is some variation in expression from pedigree to pedigree and within pedigrees, later manifestations include features similar to Parkinson's disease, limitation of eye movements, muscular fasciculations, loss of lower limb reflexes, nystagmus, tremors and diabetes mellitus. Autopsies have revealed loss of neurons and gliosis in the substantia nigra, pontine nuclei, the nuclei of the vestibulor and cranial nerves and the anterior horn.

A second example, equally well established genetically, is amyloidosis V, Finland-Type Amyloidosis or Meretoja Type Amyloidosis (Meretoja *et al.*, 1978; McKusick,

1986). An interesting case report (Sack *et al.*, 1981) described a proband of Scotch and Irish background who was apparently well until the age of about 56, when he noted some paralysis of the lower lip — surely a most unusual early sign of aging. The lip became progressively more protruding and everted until almost all of the lower gingival margin became exposed. He complained of drooling, inability to eat soup, a speech impediment and difficulty swallowing and noted occasional involuntary twitching of his right cheek. At the age of 62, an ophthalmologist noted a fine lattice-like network of densities in the cornea. Other developments included weakness of the rectus muscles and a cardiac sinus bradycardia. His muscular weakness progressed slowly and he died at the age of 79 as a result of a fall. His mother had died at age 82 following surgical repair of her drooping lower lip and face. His son was discovered to have the lattice corneal dystrophy characteristic of this disorder at the age of 43. These reproducible degenerative pathologies are attributable to an unusual propensity of these subjects to accumulate (in particular tissues) a particular variety of a class of highly insoluble proteins called amyloids. Could the coupling with aging of such conditions be related to a basic age-related decline in protein turnover (Gracy *et al.*, 1985)?

A third example, although not nearly as well established genetically, but consistent with an autosomal dominant mutation, is of special interest because of the existence of a biochemical lead, taurine deficiency. Six affected individuals were observed in three generations, with onset of symptoms late in the fifth decade (Perry *et al.*, 1975). The earliest manifestation was a depressive illness that did not respond to either antidepressant drugs or to electroconvulsive therapy. Sleep disturbance, numbness and severe weight loss were followed, during the terminal phase of life, by Parkinsonism and respiratory failure. Taurine was diminished in plasma, cerebrospinal fluid and in all regions of the brain. Taurine appears to have important antioxidative effects (Wright *et al.*, 1986). Could the coupling of aging in this disorder be related to the free radical theory of aging (Harman, 1956; Balin, 1982)?

Spontaneous genetic variation in man can identify loci of relevance to usual patterns of aging

Most physicians can agree on a set of features that characterize aging as it occurs in *most* of us. Even laymen appreciate the common observations of thinning and loss of hair, wrinkling of skin, loss of visual and auditory acuity, ocular cataracts, muscular weakness, hardening of the arteries, varicose veins, some increased propensity to fall and, especially in women, to sustain fractures of the hip, and, in many elderly individuals, some loss of short-term memory. These and certain other aspects of what could be called the "senescent phenotype" are clearly subject to some degree of control by a very large member of genes (Martin, 1978). Such experiments of nature thus can provide special opportunities to explore potential pathology of many different aspects of "ordinary" aging at the biochemical and genetic levels. Some constitutional genetic factors appear to influence the development of multiple aspects of this phenotype. They are responsible for "segmental progeroid syndromes" (Martin, 1978). Others appear to influence predominately a particular aspect of the phenotypes ("unimodal progeroid syndrome") (Martin, 1982); an example of such a gene may be the locus on chromosome 21 that greatly influences the propensity to develop the set of neuropathologies characteristic of dementia of the Alzheimer type (St. George-Hyslop *et al.*, 1987). That pathology includes a specific type of amyloid that accumulates in the brains of a variety

of aging mammalian species (Selkoe *et al.*, 1987). Thus, the locus coding for that amyloid may also be regarded as a constitutional genetic marker of aging. That locus, incidentally, also maps to chromosome 21, but there is no compelling evidence that it is identical to the major susceptibility locus identified by St. George-Hyslop *et al.* (1987).

Research on putative unimodal progeroid syndrome, such as that associated with Alzheimer's disease, is of special importance to gerontology because it has the potential to isolate gene action as regards its role in a very specific subset of the bewildering variety of alterations that characterizes aging. Such focus is more likely to lead to more rapid and significant progress on what I regard as the major mission of geriatric medicine — the identification of particular susceptibility patterns in particular individuals and the development of rational strategies of prevention and management tailored to the needs of these individuals. Especially promising, I believe, is the possibility that, for certain of these relevant genetic loci, there is expression in cultivated somatic cells, such as skin karatinocytes and fibroblasts, lymphoblastoid cell lines, glial cells, retinal epidermal cells, vascular smooth muscle cells, etc. There have been impressive advances in the technology of cell culture, permitting the cultivation of an increasing variety of cell types, sometimes in medium that is almost completely chemically defined.

Experimental systems for the identification of constitutional genetic factors that influence aging

Rather than simply relying on experiments of nature to produce genetic variation, gerontologists should be taking advantage of the many new and powerful systems that permit the experimental induction of relevant genetic variants. Many of the best such systems are nonmammalian. Such models are certainly appropriate to address many, although not all genetically relevant gerontology questions. A beautiful example of the power of this approach is given by the work of Tom Johnson with the roundworm *Caenorhabditis elegans* (Johnson, 1987). Unfortunately, however, we still know very little about the physiologic, anatomic, pathologic, biochemical and microbiological aspects of aging in that organism. It is very important that a great deal of detailed phenotypic information be accumulated. Otherwise it is possible that genetic control of enhanced longevity might in some cases result from a comparatively trivial effect — e.g., the enhanced resistance to a ubiquitous microbial agent.

A number of promising mammalian systems are emerging, however. In some instances, viable interspecific hybrids have been obtained (Dain, 1980; Rumpler and Dutrillaux, 1980; Rossant *et al.*, 1983; Rong *et al.*, 1985). The construction of transgenic mice has now become common place (Palmiter and Brinster, 1986). These protocols can obviously be used for the assessment of dominant genes, although there are complications related, e.g., to variations in the copy number, the site(s) of intregration within the genome and the physiology of expression (Palmiter and Brinster, 1986).

The use of cultivated multipotent embryonal stem cells for the isolation of mutants and for the transfection of new genetic material may provide even greater opportunities for the engineering of the genome of mammals. For practical reasons, this approach has been essentially confined to *Mus musculus* (Robertson, 1986), but it could, in principle, be applied to other mammals for the generation of chimeric and transgenic animals. *Mus musculus*, however, is clearly the mammal of choice in any systematic genetic analysis of aging in mammals. In fact, we could undoubtedly learn a great deal from

research with that species using already established less esoteric technologies, such as selective breeding (Takeda *et al.*, 1981), germ-line mutagenesis (Feinstein *et al.*, 1964; Feinstein *et al.*, 1966) and the development of recombinant inbred lines (Bailey, 1971; Taylor, 1978). A program of germ-line mutagenesis to discover relevant chromosomal and single-gene mutations in mice would of necessity be a large scale and expensive undertaking. It should be focused upon increasing the yield of variants for phenotypes of very special interest to gerontologists. While such a program might be premature at the time of this writing, it may become a viable option in another decade or so.

Genetic concomitants of speciation: Relevance for gerontology

Perhaps the most basic approach to the analysis of constitutional factors influencing aging could come via the genetic analysis of speciation. When a new species evolves, it inherits a new developmental life plan. This could include a new set of life table parameters, such as the maximum life span potential. How does this come about? Presumably, the key lies largely in the specificities of new rearrangements of what are essentially the same old genetic building blocks. For example, there is little evidence for any significant changes in the molecular structures of the kinds of proteins in chimpanzees compared to humans, yet their morphologies, developmental life plans and maximum life spans are very distinct (Wilson *et al.*, 1977). This points to important roles of regulatory sequences in the genome. How do these operate to change life span? We know virtually nothing about this fascinating question. A beginning could be made by a molecular analysis of the well-established regions of chromosomal rearrangements associated with primate evolution (Dutrillaux, 1979; Dutrillaux *et al.*, 1986a, 1986b).

RECOMMENDATIONS

The first major recommendation is that a genetic analysis of aging should start with a *particularization* of the aging phenotype of interest. For example, as my colleagues and I have pointed out elsewhere (Martin *et al.*, 1985a), in his famous paper on somatic mutation, Szilard was vague about phenotypes of interest other than overall life spans, but did mention loss of visual accommodation (Szilard, 1959). Since such presbyopia may be largely explained by changes in the physical–chemical properties of the acellular portion of the lens, somatic mutational theories are unlikely to be relevant to that particular aspect of aging. Somatic mutation may be very relevant, however, to the genesis of age-related neoplasms (Martin *et al.*, 1986b).

A second recommendation is that more efforts should be directed to a genetic analysis of nonmammalian organisms that are particularly favorable for such studies, the two classical examples being *D. melanogaster* and *C. elegans*.

As regards mammalian organisms, the species of choice is clearly *Mus musculus domesticus*. There are several powerful experimental methodologies applicable to this organism including recombinant inbreds, transgenics, chimerics and interspecific hybrids. The old fashioned but still powerful methods of germ-line mutagenesis and selective breeding should not be ignored, however.

Finally, one should begin to consider the difficult task of determining the genetic basis of speciation, which has the potential for getting at the fundamental mechanisms that regulate life span potentials.

REFERENCES

BAILEY, D.W. Recombinant inbred strains. *Transplantation* **11,** 325–327, 1971.

BALIN, A.K. Testing the free radical theory of aging. In: *Testing the Theories of Aging,* Adelman, R.C. and Roth, G.S. (Editors), pp. 138–182, Chemical Rubber Press, Boca Raton, FL, 1982.

BENZER, S. Fine structure of a genetic region in bacteriophage. *Proc. Natl. Acad. Sci. USA* **41,** 344–354, 1955.

CASARETT, G.W. Similarities and contrasts between radiation and time pathology. In: *Advances in Gerontological Research,* Strehler, B.L. (Editor), Vol. 1, pp. 109–163, Academic Press, New York, 1964.

DAIN, A.R. A cytogenetic study of a Barbary sheep *(Ammotragus lervia)* X domestic goat *(Capra hercus)* hybrid. *Experientia* **36,** 1538–1560, 1980.

DAYAN, A.D. Quantitative histological studies in the aged human brain. I. Senile plaques and neurofibrillary tangles in "normal" patients. *Acta Neuropath. (Berl.)* **16,** 85–94, 1970.

DOBZHANSKY, T. *Genetics and the Origin of Species,* 3rd Ed. revised, Columbia University Press, New York, 1951.

DUTRILLAUX, B. Chromosomal evolution in primates: tentative phylogeny from *Microcebus murinus* (Prosimian) to man. *Hum. Genet.* **48,** 251–314, 1979.

DUTRILLAUX, B., COUTURIER, J., SABATIER, L., MULERIS, M., and PRIEUR, M. Inversions in evolution of man and closely related species. *Ann. Genet.* **29,** 195–202, 1986a.

DUTRILLAUX, B., COUTURIA, J., MULERIS, M., RUMPLES, Y., and VIEGU-PEQUIGNOT, E. Relations chromosomique entre sous-ordres et infra-ordres et schema evolutif general des primates. *Mammalia* (special issue), 108–123, 1986b.

DYKES, D.D., MILLER, S.A., and POLESKY, H.F. Distribution of 1-antitrypsin variation in a US white population. *Hum. Hered.* **34,** 308–310, 1984.

EPSTEIN, C.J. Werner's syndrome and aging: A reappraisal. In: *Werner's Syndrome and Aging,* Salk, D., Fujiwara, Y., and Martin, G.M. (Editors), *Adv. Exp. Med. Biol.* **190,** 219–227, 1985.

EPSTEIN, C.J., MARTIN, G.M., SCHULTZ, A., and MOTULSKY, A.G. Werner's syndrome. A review of its symptomatology, natural history, pathologic features, genetics and relationship to the natural aging process. *Medicine* **45:** 177–221, 1966.

FAGERHAL, M.K. and COX, D.W. The Pi polymorphism: Genetic, biochemical and clinical aspects of human alpha-l-antitrypsin. *Adv. Hum. Genet.* **11,** 1–62, 1981.

FEINSTEIN, R.N., HOWARD, J.B., BRAUN, J.T., and SEAHOLM, J.E. Acatalasemic and hypocatalasemic mouse mutants. *Genetics* **53,** 923–933, 1966.

FEINSTEIN, R.N., SEAHOLM, J.E., HOWARD, J.B., and RUSSELL, W.L. Acatalasemic mice. *Proc. Natl. Acad. Sci. USA* **52,** 661–662, 1964.

GADEK, J.E. and CRYSTAL, R.G. α-1-antitrypsin deficiency. In: *The Metabolic Basis of Inherited Disease,* Stanbury, J.B., Wyngaarden, J.B., Fredrickson, D.J., Goldstein, J.L., and Brown, M.J. (Editors), pp. 1450–1467, McGraw–Hill, New York, 1982.

GEDDES, D.M., WEBLEY, M., and BREWERTON, D.A. α-1-antitrypsin phenotypes in fibrosing alveolitis and rheumatoid arthritis. *Lancet* **I,** 1049, 1977.

GRACY, R.W., YÜKSEL, K.U., CHAPMAN, M.L., CINI, J.K., JAHANI, M., LU, H.S., ORAY, B., and TALENT, J.M. Impaired protein degradation may account for the accumulation of "abnormal" proteins in aging cells. In: *Modifications of Proteins During Aging,* Adelman, R.C. and Dekker, E.E. (Editors), *Modern Aging Res.* Vol. 7, pp. 1–18, Alan R. Liss, New York, 1985.

HARMON, D. Aging: A theory based on free radical and radiation chemistry. *J. Gerontol.* **11,** 298–300, 1956.

JOHNSON, T.E. Aging can be genetically dissected into component processes using long-lived lines of *Caenorhabditis elegans. Proc. Natl. Acad. Sci. USA* **84,** 3777–3781, 1987.

MANN, D.M.A. and SINCLAIR, K.G.A. The quantiative assessment of lipofuscin pigment, cytoplasmic RNA and nucleolar volume in senile dementia. *Neuropath. Appl. Neurobiol.* **4,** 129–135, 1978.

MARTIN, G.M. Genetic syndromes in man with potential relevance to the pathobiology of aging. In: *Genetic Effects on Aging,* Bergsma, D. and Harrsion, D.E. (Editors), Birth Defects: Original Article Series, Vol. 14, No. 1, pp. 5–39, Alan R. Liss, New York, 1978.

MARTIN, G.M. Proliferative homeostasis and its age-related aberrations. *Mech. Ageing Dev.* **9,** 385–391, 1979.

MARTIN, G.M. Syndromes of accelerated aging. *Natl. Cancer Inst. Monogr.* **60,** 241–247, 1982.

MARTIN, G.M. Overview of the pathobiology of aging. In: *Interrelationship among Aging, Cancer and Differentiation*, Pullman, B., Ts'o P.O.P. and Schneider, E.L. (Editors), pp. 23–34, D., Reidel Publishing Company, Dordrecht, Holland, 1985.

MARTIN, G.M. Interactions of aging and environmental agents: The gerontological perspective. In: *Environmental Toxicity and the Aging Process*, Baker, S.R. and Rogul, M. (Editors), pp. 25–80, Alan R. Liss, New York, 1987.

MARTIN, G.M., FRY, M., and LOEB, L.A. Somatic mutation and aging in mammalian cells. In: *Molecular Biology of Aging: Gene Stability and Gene Expression*, Sohal, R.S., Birnbaum, L.S., and Cutler, R.G. (Editors), pp. 7–21, Raven Press, New York, 1985a.

MARTIN, G.M., SMITH, A.C., KETTERER, D.J., OGBURN, C.E., and DISTECHE, C.M. Increased chromosomal aberrations in first metaphases of cells isolated from the kidneys of aged mice. *Israel J. Med. Sci.* **21**, 296–301, 1985b.

McKUSICK, V.A. *Mendelian Inheritance in Man*, 7th Ed., The Johns Hopkins University Press, Baltimore, 1986.

MERETOJA, J., HOLLMEN, T., MERETOJA, T., and PENTTINEN, R. Partial characterization of amyloid proteins in inherited amyloidosis with lattice corneal dystrophy and in secondary amyloidosis. *Med. Biol.* **56**, 17–22, 1978.

MORIMATSU, M., HIRAI, S., MORAMATSU, A., and YOSHIKAWA, M. Senile degenerative brain lesions and dementia. *J. Am. Geriatr. Soc.* **23**, 390–406, 1975.

NUKIWA, T., BRANTLY, M., GARVER, R., PAUL, L., COURTNEY, M., LE COCQ, J-P., and CRYSTAL, R.G. Evaluation of "at risk" alpha l-antitrypsin genotype SZ with synthetic oligonucleotide gene probes. *J. Clin. Invest.* **77**, 528–537, 1986.

PALMITER, R.D. and BRINSTER, R.L. Germ-line transformation of mice. *Ann. Rev. Genet.* **20**, 465–499, 1986.

PERRY, T.L., BRATTY, P.J.A., HANSEN, S., KENNEDY, J., URQUHART, W., and DOLMAN, C.L. Hereditary mental depression and Parkinsonism with taurine deficiency. *Arch. Neurol.* **32**, 108–113, 1975.

ROBERTSON, E.J. Pluripotential stem cell lines as a route into the mouse germ line. *Trends Genet.* **2**, 9–13, 1986.

RONG, R.H., CAI, H., YANG, X., and WEI, J. Fertile mule in china and her unusual foals. *J. Roy. Soc. Med.* **78**, 821–825, 1985.

ROSSANT, J., CROY, B.A., CLARK, D.A. and CHAPMAN, V.M. Interspecific hybrids and chimeras in mice. *J. Exp. Zool.* **228**, 223–233, 1983.

RUMPLER, Y. and DUTRILLAUX, B. Chromosomal evolution in Malagasy lemurs. V. Chromsomal banding studies of *Lemur fulvus albifrons*, *Lemur rubriventer* and its hybrids with *Lemur fulvus fulvus*. *Folia Primatol. (Basel)* **33**, 253–261, 1980.

SACK, G.H., JR., DUMARS, K.W., GUMMERSON, K.S., LAW, A., and McKUSICK, V.A. Three forms of dominant amyloid neuropathy. *Johns Hopkins Med. J.* **149**, 239–247, 1981.

SANDBERG, A.A. and WAKE, N. Chromosomal changes in primary and metastatic tumors and in lymphoma: their nonrandomness and significance. In: *Genes, Chromosomes and Neoplasia*, Arrighi, F.E., Rao, P.N. and Stubblefield, E. (Editors), pp. 297–333, Raven Press, New York, 1981.

SELKOE, D.J., BELL, D.S., PODLISNY, M.B., PRICE, D.L., and CORK, L.C. Conservation of brain amyloid proteins in aged mammals and humans with Alzheimer's disease. *Science* **235**, 873–877, 1987.

ST. GEORGE-HYSLOP, P.H., TANSI, R.E., POLINSKY, R.J., HAINES, J.L., NEE, L., WATKINS, P.C., MYERS, R.H., FELDMAN, R.G., POLLEN, D., DRACHMAN, D. *et al.* The genetic defect causing familial Alzheimer's disease maps on chromosome 21. *Science* **235**, 885–890, 1987.

SZILARD, L. On the nature of the aging process. *Proc. Natl. Acad. Sci. USA* **45**, 30–45, 1959.

TAKEDA, T., HOSOKAWA, M., TAKESHITA, S., IRINO, M., HIGUCHI, K., MATSUSHITA, T., TOMITA, Y., YASUHIRA, K., HAMAMOTO, H., SHIMAZU, K., ISHII, M., and YAMAMURO, T. A new murine model of accelerated senescence. *Mech. Ageing Dev.* **17**, 183–194, 1981.

TAYLOR, B.A. Recombinant inbred strains: use in gene mapping. In: *Origin of Inbred Mice*, Morse, H.C. III (Editor), pp. 423–438, Academic Press, New York, 1978.

VOGEL, F. and MOTULSKY, A.G. *Human Genetics*, 2nd Ed., Springer, New York, 1987.

WARNER, H.R., BUTLER, R.N., SPROTT, R.L., and SCHNEIDER, E.L. *Modern Biological Theories of Aging*. Raven Press, New York, 1987.

WILSON, A.C., WHITE, T.J., CARLSON, S.S., and CHERRY, L.M. Molecular evolution and cytogenetic evolution. In: *Molecular Human Cytogenetics,* Sparkes, R.S., Comings, D., and Fox, C.F. (Editors), ICN-UCLA *Symposia on Molecular and Cell Biology,* Vol. 7., pp. 375–393, Academic Press, New York, 1977.

WRIGHT, C.E., TALLAN, H.H., LIN, Y.Y., and GAULL, G.E. Taurine: biological update. *Ann. Rev. Biochem.* **55,** 427–453, 1986.

YUNIS, J.J. Chromosomal rearrangements, genes, and fragile sites in cancer: clinical and biologic implications. In: *Important Advances in Oncology,* De Vita, V.T., Jr., Hellman, S., and Rosenberg, S.A. (Editors), pp. 93–128, J.B. Lippincott, Philadelphia, 1986.