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Molecular biology of brain aging and neurodegenerative disorders

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Abstract. A significant component of the aging process is genetically determined. Numerous theories of aging exist, many of which postulate the existence of "longevity genes." Recent advances in molecular biological and other techniques have allowed a significantly greater understanding of aging and age-related disease. This will be illustrated by four genetic and sporadic diseases: Alzheimer's disease (AD) and related disorders, transthyretin dementia, cerebral amyloid angiopathy-Icelandic type and scrapie related diseases. Alzheimer's disease (AD), the most common of this group, is the leading cause of dementia in Western countries. Recent genetic and biochemical studies have shown the involvement of at least four genes in the pathogenesis of AD. In early-onset familial AD mutations in the βPP, S182 (presenilin 1) and STM2 (presenilin 2 or E5-1) genes have been found, while in the more common late-onset AD the presence of the apolipoprotein E4 isotype is a major risk factor. Genetic studies have also helped to elucidate the etiology of rarer cerebral amyloidoses such as the recently described Hungarian amyloidosis that is characterized by meningocerebrovascular amyloid deposition, with resultant dementia. This disease is linked to a mutation in the transthyretin gene. It is hoped that in the near future this increase in knowledge will allow the development of therapeutic approaches to slow the aging process.

Review

Key words: aging Alzheimer's diesease, amyloid β, S182, Hungarian amyloidoisis, transthyretin, prion

INTRODUCTION

Aging is a process that concerns us all. The increasing armamentarium provided by molecular biology and other techniques has allowed greater understanding of some of the processes involved. Numerous definitions of aging exist; an acceptable and commonly used definition is that aging is the total of all changes an organism undergoes from its conception to its death, including development, maturation and adulthood. Since the life span varies between and within species and human longevity is partially hereditary, it is clear that genetic factors influence the aging process (Johnson 1993, Schacter et al. 1993, Vijg et al. 1995). A number of studies have indicated that there is a heritable component to the human span. An initial work in 1934 was the first to clearly show a correlation of the life span in a group of nonagerians with the long-lived phenotype of their ancestors (Pearl et al. 1934). In a more recent study of 7,000 offspring of nonagerians it was shown that longer-lived parents had longer--lived children (Abbot et al. 1978). Furthermore in twin studies a higher concordance of ages of death was found in monozygotic *versus* dizygotic twins (Kallman et al. 1948). However, it is a commonly accepted hypothesis that aging is a multifactorial process. An individual's longevity is dependent on genetic background, environmental factors and interactions between the two.

THEORIES OF AGING

Over 300 different theories of aging have been proposed (Johnson 1993, Schächter et al. 1993, Vijg et al. 1995), many of which overlap or are similar. Some of them are outlined in Table I.

A commonly cited theory of aging that ties together many scattered observations is the disposable soma theory (Kirwood et al. 1991, Schächter et al. 1993). The underlying notions of this theory are that: (1) with the exception of humans, most death in natural populations occurs from accidental causes and is not related to aging; (2) survival is dependent on somatic maintenance. These housekeeping functions are energetically costly; (3) it is wasteful and therefore disadvantageous for an organism to invest a greater proportion of metabolic resources to long-term survival than is needed for the organism to function in good order through its naturally expected life in the wild. From these statements it follows that the optimum investment in somatic maintenance is going to be below the hypothetical threshold necessary for indefinite survival; hence, aging is an evolutionary necessity.

TABLE I

Theories of Aging

- Organismic level
  - ♦ Disposable soma theory
- Organ level
  - ♦ Neuroendocrine theories
  - ♦ Immunological theories
- Cellular Level
  - ♦ Intrinsic cellular aging
    - ♦ Hayflick phenomena of proliferative senescence
    - ♦ Apoptosis
  - ♦ Free radical theory
  - ♦ Protein error and post-translational modification theory
  - ♦ DNA mutation theories
    - ♦ Somatic DNA

To account for the known genetic influences on longevity, one would expect the existence of genes which may regulate the amount of cellular energy devoted to repair of free radical damage, protein transcription errors, post-translational modifications of proteins (such as glycation) and DNA mutations that accumulate with time. A number of genetic approaches exist to identify such postulated "longevity genes."

GENETIC APPROACHES TO STUDY LONGEVITY

Several different methods can be used to identify genes that influence the human life-span. One of these is "case control" studies (Cox et al. 1989). In the latter, allele and genotype frequencies at poly-

morphic marker loci are compared between a long--lived group and a control group. If statistically significant differences are found, this will support that the marker polymorphism has an influence on longevity or more likely that it is in linkage disequilibrium with other genes that play such a role. An example of a recent study which used this approach, compared a population of 338 centenarians with a control group and found that the apolipoprotein (apo) E4 allele of apoE was decreased in the long--lived group and that the E2 allele was increased (Schächter et al. 1994). These differences could be related to the impact of apoE4 on the risk of cardiovascular disease and Alzheimer's disease (AD) and the protective effect of apoE2 in AD.

Another possible strategy is the use of sibling pair analysis (Blackwelder et al. 1985). This method depends on the non-random segregation of particular marker loci in long-lived siblings. If a polymorphism that is linked to the marker increases longevity it will be detected by a shift in its distribution among long-lived siblings.

Genetic approaches have also been applied with greater success to the elucidation of age-related disease. This has used a positional cloning technique (Orr et al. 1995). In recent years numerous highly informative, DNA polymorphic markers have been identified, with the result that monogenic traits or

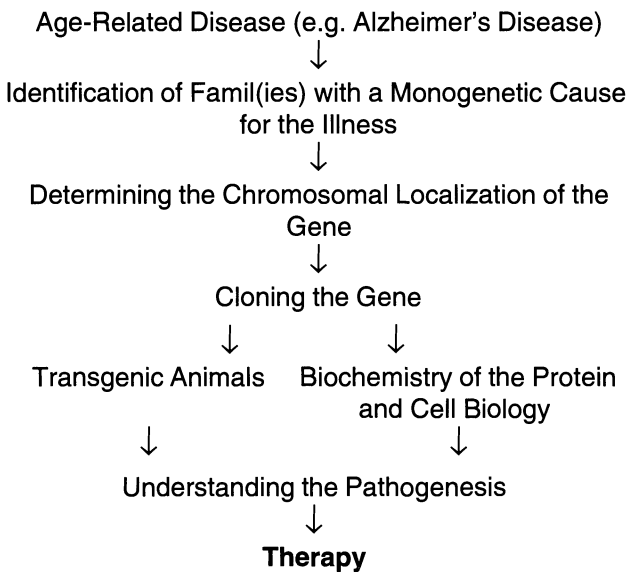

Fig. 1. Positional cloning of an age-related gene.

disorders can be localized to precise regions of a human chromosome. This mapping of a gene is the first step for its subsequent isolation and cloning as depicted in Fig. 1. Essential for this first step is the identification of families with a single genetic cause for their age-related illness. This may be very complicated as a particular disease phenotype may be genetically or etiologically heterogenous.

This approach consists of genetic strategies that localize a gene to within a small chromosomal segment of approximately 1 to 2 megabases of DNA. When this is accomplished, the chromosomal segment is cloned, typically in overlapping clones of human DNA in yeast artificial chromosome (YAC). These YACs are in turn screened for genes that may contain a mutation in the disease-causing gene. However as the Human Genome Project proceeds the above approach of positional cloning will be overtaken by a positional candidate method (Ballabio 1993, Collins 1995). This would combine linkage analysis, similar to the first part of positional cloning, followed by an analysis for mutations of candidate genes that have been previously suggested to map to that locus.

AGE-RELATED DISEASE

A significant group of age-related diseases are the cerebral amyloidoses (Ghiso et al. 1994, Wisniewski et al. 1994b), where great strides were recently made in identifying the genes associated with these diseases. The amyloidoses are an etiologically diverse group of diseases characterized by the deposition of fibrillar protein (amyloid) mainly in the extracellular space of different tissues, leading to cell damage, organ dysfunction and death. The most common of these are the cerebral amyloidoses listed in Table II.

Aβ AND RELATED AMYLOIDOSES

Alzheimer's disease (AD) is the most common form of human amyloidosis, and is the major cause of dementia, affecting more than 5% of the popula-

TABLE II

Cerebral Amyloidoses

Disease	Major Amyloid Proteins	Amyloid Precursor Protein	Linked Genes
Early-onset Alzheimer's disease (<60 yrs)	Aβ	β-amyloid precursor protein	βPP, S182, STM2 variants
Late-onset Alzheimer's disease (>60 yrs)	Aβ	β-amyloid precursor protein	Apolipoprotein E4
Down's syndrome	Aβ	β-amyloid precursor protein	βPP
*HCHWA-Dutch	Aβ	β-amyloid precursor variant	βPP variants
Sporadic cerebral amyloid angiopathy	Aβ	β-amyloid precursor proteins	Apolipoprotein E4
*HCHWA-Icelandic	ACys	Cystatin C variant	Cystatin C variant
Hungarian amyloidosis	ATTR	Transthyretin (TTR) variant	TTR variant
Creutzfeldt-Jacob disease	APrP	Protease Resistant Protein (PrP) and variants	PRNP and PRNP variants
Gerstmann-Sträussler-Scheinker disease	APrP	PRP variants	PRNP variants
Kuru	APrP	PRP	-
British Amyloidosis	?	?	

*HCHWA: Hereditary Cerebral Hemorrhage with Amyloidosis

tion over the age of 65 (Ghiso et al. 1994, Wisniewski et al. 1994b). AD has become a huge socioeconomic problem with the "graying" of Western societies. Neuropathologically, AD is characterized by three major lesions: (1) intraneuronal, cytoplasmic deposits of neurofibrillary tangles (NFT), (2) neuritic or senile plaques, (3) cerebrovascular amyloidosis (Ghiso et al. 1994, Wisniewski et al. 1994b). The major, but not the only, component of the last two lesions is amyloid β (Aβ). This peptide is 39 to 44 residues long with heterogenous amino and carboxyl termini (Glenner et al. 1984, Masters et al. 1985, Selkoe et al. 1986, Prelli et al. 1988, Roher et al. 1993, Wisniewski et al. 1994). Aβ is a fragment of a larger precursor protein (βPP) that is encoded by a large single gene on chromosome 21 (Goldgaber et al. 1987, Kang et al. 1987, Robakis et al. 1987, Tanzi et al. 1987). This gene contains at least 19 exons, spanning more than 190 kilobases, with more than 10 isoforms of βPP mRNA that can be generated by alternative splicing (Kitaguchi et al. 1988, Prelli et al. 1988, Tanzi et al. 1988, DeSauvage et al. 1989, Leimaire et al. 1989, Golde et al. 1990. Yoshikai et al. 1990, Konig et al. 1992). βPP has a predicted structure of a multidomain, transmembrane cell-surface receptor (Goldgaber et al. 1987, Kang et al. 1987, Robakis et al. 1987, Tanzi et al. 1987). The Aβ sequence arises from portions of exons 16 and 17 (using βPP770 numbering); therefore, Aβ cannot be generated by alternative splicing of βPP but requires proteolytic cleavage at both its N- and C-termini. One of the first discovered metabolic processing pathways of βPP to its shorter fragments involves a cleavage at Aβ residue 16 by the so-called α-secretase, releasing a large soluble protein containing only the N-terminal sequence of Aβ (Esch et al. 1990, van Nostrand et al. 1990, Sisodia et al. 1990, Wang et al. 1991, Ramabhadran et al. 1993). Studies in the last three years have documented the presence of a soluble Aβ-like peptide (sAβ) in normal biological fluids at low concentrations (Haass et al. 1992, Seubert et al. 1992, Shoji et al. 1992, Busciglio et al. 1993). Amino acid sequence analysis indicates that the major sAβ species

is Aβ 1-40, homologous to the major amyloid protein from cerebrovascular lesions. However, larger sAβ species, such as Aβ 1-42 and shorter ones (Aβ 1-28) also exist (Vigo-Pelfrey et al. 1993). Soluble Aβ is thought to arise from βPP by the combined actions of β-secretase at the N-terminal and γ-secretase at the C-terminal.

AD is genetically heterogenous. It can be divided into early-onset (below age 60) and late-onset forms. Most early-onset cases show autosomal dominant inheritance and make up approximately 5% of all AD cases (Smith et al. 1994b, Wisniewski et al. 1994). The βPP gene was the first locus to be investigated for mutations in early-onset AD families. The first βPP mutation discovered was in hereditary cerebral hemorrhage with amyloidosis, Dutch type (HCHWA-D), or the Dutch variant of AD (Levy et al. 1989). This condition is characterized by the deposition of amyloid mainly in cerebral vessels, with a halo of Aβ immunoreactivity in the parenchyma surrounding many of these vessels (Timmers et al. 1990, Wisniewski et al. 1992a). HCHWA-D is associated with a glutamine substitution for glutamic acid at codon 693 corresponding to residue 22 of Aβ (Levy et al. 1990). Since that time a number of pathogenic βPP mutations have been discovered, which are listed in Table III.

How the known βPP mutations are associated with AD pathology is under intensive current study. A number of *in vitro* studies use peptides homologous to Aβ and containing the Dutch variant mutation have shown that the presence of the Glu at residue 22 accelerates or stabilizes fibrillogenesis (Wisniewski et al. 1991, Clements et al. 1993). Hence this may promote the conversion of the normal sAβ into Aβ deposited in brain parenchyma. On the other hand the Swedish family with the double mutation at codons 670/671 have been shown to have a slightly increased level of sAβ (Citron et al. 1992, Citron et al. 1993), while families with codon 717 mutation have been shown to have increased production of longer variants of sAβ such as Aβ 1-42 vs. the more soluble Aβ 1-40 (Suzuki et al. 1994). Whether these findings or other unknown factors are important in the pathogenesis of AD in these cases remains to be determined.

A potential advance in understanding the role of βPP mutations and the pathogenesis of AD in

TABLE III

βPP Mutations

Codon	Amino Acid Substitution	Phenotype	Cases Described	References
670/671	Lys→Asn/ Met→Leu	AD	1 Swedish family	(Mullan et al. 1992)
673	Ala→Thr	--	1 case of stroke and myocardial infarction	(Peacock et al. 1993)
692	Ala→Gly	AD and cerebral hemorrhage	1 Dutch family	(Hendriks et al. 1992)
693	Glu→Gln	HCHWA-D	3 Dutch families	(Levy et al. 1990)
693	Glu→Gly	--	1 case of AD	(Kamino et al. 1992)
713	Ala→Val	--	1 case of schizophrenia	(Jones et al. 1992)
713	Ala→Thr	--	1 case of AD and several controls	(Carter et al. 1992)
717	Val→Ile	AD	3 English families, 2 Japanese	(Fidani et al. 1992)
717	Val→Phe	AD	1 American family	(Murrell et al. 1991)
717	Val→Gly	AD	1 English family	(Chartier-Harlin et al. 1991)

general has been the development of a transgenic mouse model that uses a construct of the full-length human βPP complementary DNA with the βPP 717 Val to Phe mutation under the control of the platelet-derived growth factor promoter (Games et al. 1995). Inserted into this cDNA construct were introns 6, 7 and 8 from the βPP gene, which are important for alternative splicing of the gene product. These mice, after the age of 6 to 9 months, began to exhibit deposits of human Aβ in the parenchyma of the hippocampus, corpus callosum and cerebral cortex. Some of these deposits are Congo red positive and therefore amyloid. These mice have not, so far, developed NFT or congophilic angiopathy; however, they may provide an important tool to study the pathogenesis of AD.

Most cases of early-onset AD do not have any of the βPP mutations. Seventy to 80% of these families are linked to a locus on chromosome 14 (Van-Broeckhoven 1995). Recently the gene at this locus has been cloned, and named S182 or presenilin 1 (Sherrington et al. 1995). The primary structure of the longest open reading frame predicts a 467 amino acid protein that contains seven hydrophobic, putative membrane spanning domains (see Fig. 2). This predicted structure of the S182 protein is consistent with a receptor, a channel protein or a structural membrane polypeptide. Already 20 different mutations have been reported in the S182 gene in early-onset AD kindreds (Fig. 2) (Sherrington et al. 1995). Another locus linked to a Volga-German FAD kindred has recently been found on chromo-

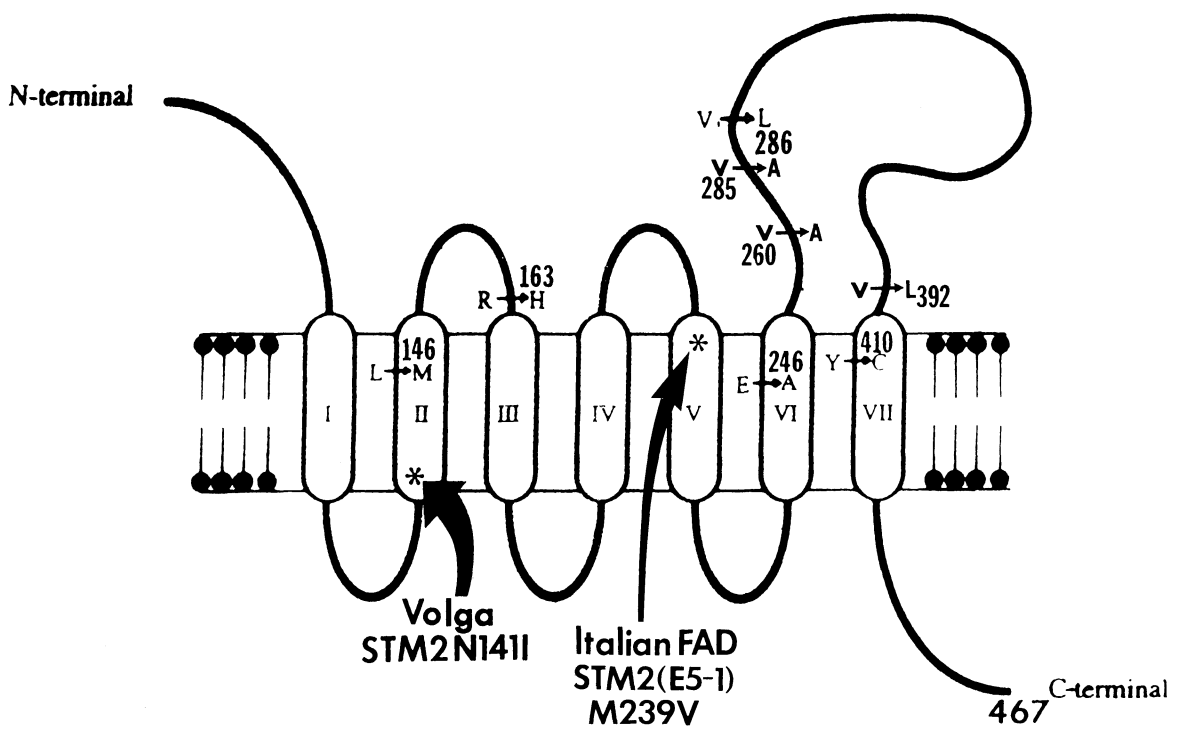

Fig. 2. This cartoon depicts the hypothetical structure of the S182 protein within the cell membrane (Sherrington et al. 1995). The S182 gene is located on chromosome 14 (Sherrington et al. 1995). The transmembrane domains are numbered I through VII. Some of the known mutations associated with early-onset familial AD and their approximate locations are shown by the horizonal arrows (Rogaev et al. 1995, Sherrington et al. 1995). The wild-type sequence is on the right of the horizonal arrows. In addition the location of the two mutations found in the STM2 (or E5-1) gene (that is homologous to S182 but is located on chromosome 1) is indicated by asterisks and vertical arrows (Levy-Lahad et al. 1995, Rogaev et al. 1995). The first of these to be described was in the Volga kindred of FAD (Levy-Lahad et al. 1995), (STM2, N141 I) and the next was described in an Italian kindred (STM2 M239V) (Rogaev et al. 1995).

some 1 (Levy-Lahad et al. 1995, Rogaev et al. 1995). This gene has also been cloned and is named STM2 (the second seven transmembrane gene associated with AD) (Levy-Lahad et al. 1995) or E5-1 (Rogaev et al. 1995). STM2 encodes for a protein predicted to have 554 amino acids. At the primary structure level STM2 and S182 are 67% identical over all, while in the hydrophobic transmembrane regions they are 84% identical (Levy-Lahad et al. 1995). A single mutation at codon 141 (N141I) has been found in the Volga kindred in the STM2 gene (Levy-Lahad et al. 1995), while another mutation in the STM2 gene has been found in an Italian FAD kindred at codon 239 (M239V) (Rogaev et al. 1995). Little is known about the function of these closely related genes, and whether they interact with βPP, Aβ or tau. The most homologous protein to the S182 gene product has been fount to be sel-12 (Levitan et al. 1985). Sel-12 is approximately 48% homologous with S182 and appears to facilitate signaling mediated by lin-12 and glp-1, which are receptors for intercellular signals that specify cell fate in Caenorhabditis elegans. It is possible that S182 has a similar function given the degree of homology. Whether the variant S182 and STM2 proteins result in a disturbance of cellular trafficking that is involved in AD remains to be determined. Also a crucial question will be whether the S182 and STM2 genes play a role, not only in the relatively rare early-onset FAD cases but also in the pathogenesis of AD in general. Our recent immunohistochemical studies have found a carboxyl termial epitope of S182 to be found in neuritic plaques and congophilic vessels of both chromosome 14 linked AD and sporadic cases (Wisniewski et al. 1995b and unpublished observations) consistent with a more general role for the S182 and related genes.

Most AD cases are late-onset in type. A major genetic risk factor which has been identified for this most common form of AD is the presence of the apolipoprotein (apo) E4 allele (Weisgraber et al. 1994). In some populations it has been shown that up to 90% of individuals who are homozygous for apo E4 will develop AD if they live to the age of 80 (Corder et al. 1993). One to two percent of the population is homozygous for apo E4 and 15 to 20% are heterozygous (Weisgraber et al. 1994). Apo E4 differs from apo E3 by a single amino acid substitution at reside 112; apo E4 has an arginine instead of cysteine. Whether apo E4 has a direct role in late-onset AD is know known. We have suggested that apo E acts as a "pathological chaperone," inducing a β-pleated sheet structure (Wisniewski et al. 1993b). This hypothesis is supported by several *in vitro* studies (Ma et al. 1994, Wisniewski et al. 1994a). We have also shown that apo E is complexed to Aβ and co-purified with the amyloid from senile plaques. A carboxyl fragment of apo E is also able *in vitro* to form amyloid-like fibrils, raising the possibility that the amyloid in AD is heterogenous. The apo E which is found in Aβ deposits may act to augment or seed amyloid fibril formation (Wisniewski et al. 1995a).

The genetic associations with AD are diverse and include mutation of the S182, STM2 and βPP genes as well as the presence of the apo E4 allele. How these are associated with the neuronal death and reduced synaptic density that correlates best with the dementia of AD is unknown. General mechanisms which have been hypothesized to play a role in AD have included apoptosis, oxidative damage and excitotoxicity. Some evidence exists for each of these.

Apoptosis or programmed cell death is marked by a series of characteristic morphological changes, with a distinctive pattern of DNA fragmentation (Carson et al. 1993, Dickson 1995). These changes are distinct from that seen with necrosis. Some studies have indicated that Aβ peptides when in an aggregated form can induce apoptotic cell death in tissue culture (Disterhoft 1994). Calcium has been implicated as a secondary messenger in apoptosis, as well as in excitotoxicity and glutamate-mediated cell death. Several studies have suggested that aggregated or β-pleated Aβ can induce free radical production in neurones which can disrupt Ca^{2+} regulatory mechanisms, resulting in aberrant elevations of Ca^{2+} and an increased sensitivity to excitory stimuli (Disterhoft 1994). Work is ongoing in several laboratories to try to determine if these pro-

cesses can indeed be final common pathways of toxicity in AD or are only *in vitro* phenomena.

HEREDITARY CEREBRAL HEMORRHAGE WITH AMYLOIDOSIS, ICELANDIC TYPE

This is an autosomal dominant form of congophilic angiopathy that affects a number of Icelandic families, often leading to death before the age of 40 because of massive intracerebral hemorrhages (Ghiso et al. 1994). The pathological findings show cerebrovascular involvement with amyloid infiltration of the walls of small arteries and arterioles in the cerebral cortex and leptomeninges. Genetic studies have found that the Cystatin C gene in patients with HCHWA-I has a missense mutation that results in a single amino acid substitution at codon 68 (L68Q) (Levy et al. 1989). Cystatin C is normally present in cerebrospinal fluid and in other biological fluids. Patients with HCHWA-I have low levels of the variant Cystatin C protein in their CSF (Grubb et al. 1984), suggesting that the mutation favors the amyloidogenic conformation of the protein and promotes its deposition.

HUNGARIAN AMYLOIDOSIS

We have recently found a Hungarian kindred with extensive meningocerebrovascular amyloid deposits (Vidal et al. 1996). Clinically this condition is associated with a progressive dementia, cerebellar dysfunction and spasticity, with an onset in the fourth decade. Immunohistochemical studies have shown this amyloid to be transthyretin (TTR) related. Genetic analysis has found a single missense mutation in the TTR gene at codon 18 resulting in a substitution of aspartic acid to glycine

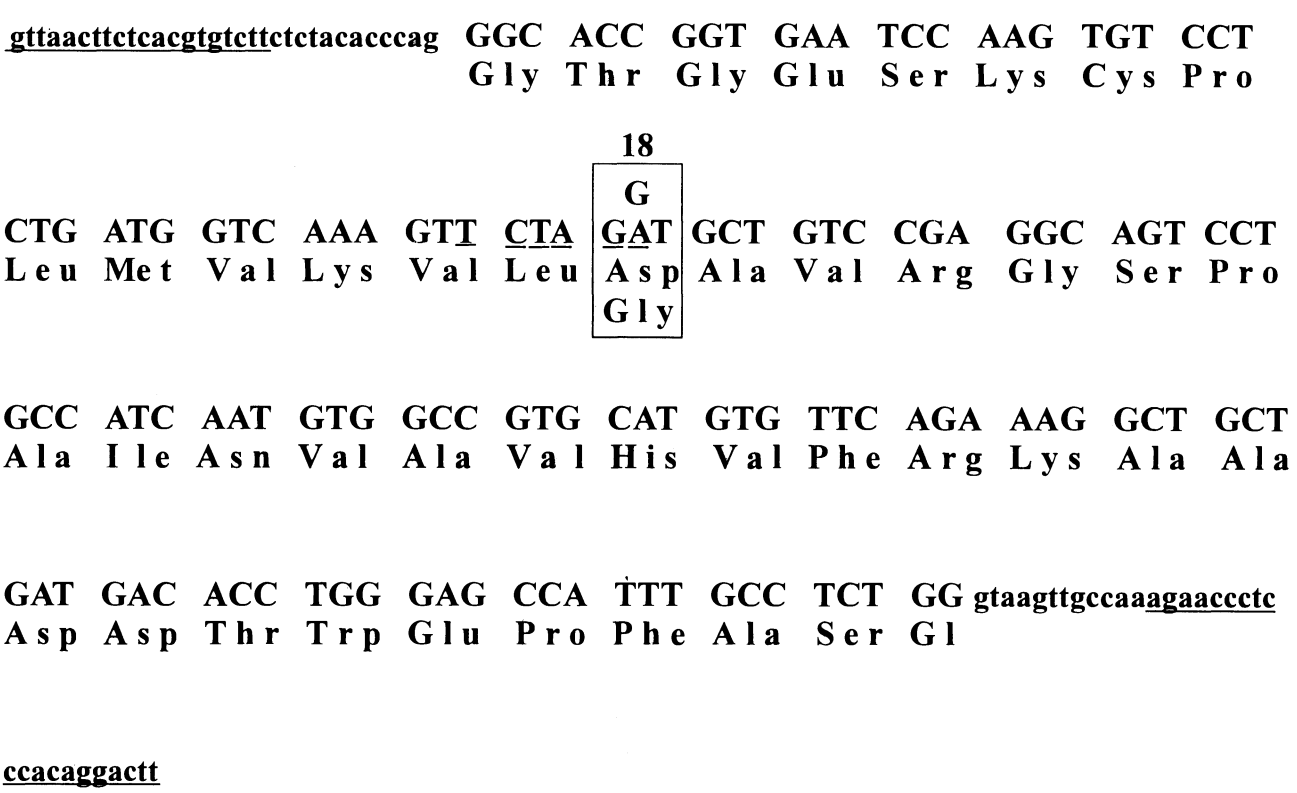

Fig. 3. Shows the DNA sequence of exon 2 of the TTR gene. The exons are in capital letters while the flanking introns are in lower case letters. At codon 18 (in the rectangle) the nucleotide substitution is shown on the top of the sequence and the amino acid substitution is shown on the bottom. The bottom line shows a XbI restriction site.

(D18G) (see Fig. 3); this mutation has never been reported in the TTR gene and has not been found in a large series of controls (Vidal et al. 1996). Over 44 pathogenic mutations have already been reported in the TTR gene (Saraiva 1995). These mutations are typically associated with familial amyloid polyneuropathy (FAP) or cardiomyopathy, with no central nervous system symptoms. In addition TTR related amyloid occurs in senile systemic amyloidosis, typically in the absence of any associated mutation. The newly recognized kindred, which we have described above, has expanded the number of proteins which can be associated with cerebral amyloidosis.

PRION-RELATED AMYLOIDOSES OR PRIONOSES

The human prionoses are a group of disorders characterized by neuronal spongiform degeneration, astrocytic gliosis, neuronal loss and in some cases parenchymal amyloid deposition (Prusiner et al. 1994, DeArmond et al. 1995). This group includes Creutzfeld-Jacob disease (CJD), Gerstmann-Sträussler-Scheinker disease (GSS) and kuru. Other human prion diseases which are not typically associated with amyloid deposits include fatal familial insomnia (FFI) and familial progressive subcortical gliosis (PSG) (Prusiner 1991, DeArmond et al. 1995, Peterson et al. 1995). The human prion disease can present as sporadic, dominantly inherited or as transmissible neurodegenerative disorders (Prusiner 1991, DeArmond et al. 1995). Approximately 10 to 15% of human prion diseases are hereditary. These have been linked to over 12 different mutations in the human prion protein gene (PRNP) on chromosome 20 (Prusiner 1991, DeArmond et al. 1995). Interestingly the newly recognized familial prion disease of PSG is not linked to mutations in the PRNP gene but to a locus on chromosome 17 (Petersen et al. 1995), suggesting that as in AD, more than one gene can be involved in the pathogenesis of this disorder.

Central to the pathogenesis of prion-related diseases is thought to be the conversion of the normal cellular PrP, designated PrP^{C}, into the protease-resistant and infectious PrP^{Sc} (Prusiner 1991, DeArmond et al. 1995). The only known post-translational modification of PrP^{C} that occurs during its transformation into PrP^{Sc} is the acquisition of a β-sheet conformation. While all the prionoses are associated with an abnormal conformation of PrP, this leads to fibrillar deposition of the PrP as amyloid only is some cases. GSS is always associated with amyloid and most cases of Kuru have amyloid deposits, while only 10% of CJD has amyloid. Hence, the prionoses can be categorized into "fibrillar" and "non-fibrillar" forms. PrP^{Sc} is 43% β-sheet and 30% α-helix, while PrP^{C} is 3% β-sheet and 42% α-helix (Pan et al. 1993, Njuyen et al. 1995). Interestingly, it has recently been shown that synthetic PrP peptides with a reduced α-helical structure, can alter PrP^{C} so that it has some of the properties of of PrP^{Sc} (Kaneko et al. 1995). This conversion of a normal soluble protein into a β-sheet, fibrillar structure in PrP related diseases is analogous to the conversion of sAβ to the amyloid deposits in senile plaques.

THERAPEUTIC AND FUTURE DIRECTIONS

Molecular biological and other techniques have allowed significant advances into the pathogenesis of age-related diseases as well as the normal aging process. Unfortunately no therapeutic interventions have arisen yet from this new information. In many cases identification of linkage with a gene in an age-related illness is only the first step in understanding how it is involved in pathogenesis. Extensive work on the biochemistry and cell biology of the involved protein is needed for development of efficacious therapeutic approaches. So far in mammals the only intervention conclusively shown to slow aging and delay the onset of age-related disease is dietary caloric restriction (Roth et al. 1995). For several decades it has been shown that laboratory rats placed on calorically restricted diets live longer, remain healthier and maintain physiological and behavioural functions better than their *ad libitum* fed counterparts (Roth et al. 1995). Currently the National

Institute on Aging is engaged in a study to confirm whether similar effects are seen in primates. It is hoped that the fast pace of accumulating knowledge into the age-related diseases will lead to other, more useful ways to slow the process. In particular in the AD research field, where in the last few months two new disease-associated genes have been identified, and a better transgenic mouse model has been developed, such a hope may be justified.

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