Published in final edited form as:
FEBS Lett. 2009 August 20; 583(16): 2663–2673. doi:10.1016/j.febslet.2009.07.031.

Animal models of human amyloidoses: Are transgenic mice worth the time and trouble?

Joel N. Buxbaum, M.D.
Department of Molecular and Experimental Medicine W.M. Keck Center for Autoimmune Diseases The Scripps Research Institute 10550 North Torrey Pines Road Mail Code MEM-230 La Jolla, CA 92037 858-784-8885 (Phone) 858-784-8891 (Fax) jbux@scripps.edu

Abstract

The amyloidoses are the prototype gain of toxic function protein misfolding diseases. As such, several naturally occurring animal models and their inducible variants provided some of the first insights into these disorders of protein aggregation. With greater analytic knowledge and the increasing flexibility of transgenic and gene knockout technology, new models have been generated allowing the interrogation of phenomena that have not been approachable in more reductionist systems, i.e. behavioral readouts in the neurodegenerative diseases, interactions among organ systems in the transthyretin amyloidoses and taking pre-clinical therapeutic trials beyond cell culture. The current review describes the features of both transgenic and non-transgenic models and discusses issues that appear to be unresolved even when viewed in their organismal context.

Keywords

amyloidosis; transgenic models; amyloid A; AL amyloid; cystatin c; gelsolin; transthyretin; Alzheimer's disease; serpinopathies; Familial dementias

Why do we need animal models of protein misfolding disorders? Historically, when it was technically difficult to study human disease in detail, except at the autopsy table, we searched for other means of obtaining useful information and many diseases were modeled (with more or less fidelity) in animals. High resolution imaging, sensitive comprehensive physiologic monitoring capability and convenient multiple detailed analyses of plasma and cellular components of the body fluids have allowed us to define many physiologic parameters in living humans regardless of their state of health. On the molecular level we can study the attributes of folding and misfolding in infinite detail using purified proteins in the test tube in an experimentally convenient time frame. We can add small molecules, peptides, and vary conditions so that we accelerate or inhibit fibril formation. We can derive mathematical models that explain why any given precursor goes from a soluble to insoluble state and determine the precise nature of that state in atomic detail. Having done all that it is still not possible to explain why an amyloidogenic protein, perhaps present from conception in roughly the same molar concentrations, does not form tissue deposits until late in life. Nor can we explain why deposits occur in some tissues and not in others and why in some people and not in others. Finally, while it is possible to expose a cell to a potential therapeutic, moving the agent to the clinic

usually requires extensive animal testing. Thus good animal models have an important place in the investigation of diseases of protein conformation.

Protein misfolding disorders come in a variety of flavors. We can describe them as genetic or acquired, intra or extracellular, gain of function (GOF) or loss of function (LOF), localized or systemic, with or without morphologically evident aggregates, neurodegenerative or not. Amyloidosis, the prototypic protein folding disorder (or group of disorders), was noted in the mid-19th century in autopsy specimens from patients with a diverse set of diseases [1]. Each amyloid represented the detectable end product of many pathologic processes culminating in a final common pathway of aggregation, fibril formation and tissue deposition, although not necessarily in that order.

Twenty seven such proteins have been identified as amyloid precursors in humans (Table 1). Further, in addition to the quantitatively dominant fibrillar component, all deposits regardless of the fibril precursor contain a set of accessory molecules including serum amyloid P-component (SAP), apolipoprotein E, and the proteoglycan perlecan. The functions of these three molecules, with respect to fibril formation, are still obscure [2]. In addition to these "accessory proteins" some deposits, particularly those in the brain (which have been studied in most detail) contain other molecules which have been hypothesized to either enhance deposition or retard the process [3].

In the two most common systemic human amyloidoses, i.e. AA and AL, large amounts of precursor protein could be obtained from human or animal body fluids and the process of fibril formation could be studied in the test tube [4;5]. The development of recombinant molecular techniques made it possible to obtain large amounts of pure precursor proteins responsible for most of the clinical amyloid syndromes for *in vitro* studies of fibrillogenesis. In addition, incubation of a number of molecules associated with neurodegenerative diseases, under amyloid fibril-forming conditions *in vitro* revealed that they too behaved as did classical amyloid precursors under the same conditions [6]. Subsequently the family of amyloid precursors was expanded to include all those proteins that formed fibrillar aggregates with the characteristic β-sheet structure and dye-binding properties found in the original extracellular disorders *in vivo* and *in vitro*.

There have been numerous studies on naturally occurring and inducible animal amyloidoses including amyloid A in a variety of species, scrapie in sheep, BSE in cattle, ApoAII isomorphs in the Senescence Accelerated Mice (SAM) and amyloid-containing insulinomas in cats [7]. To the extent that the same or similar conditions occur in humans the animal disorders can serve as disease models.

Murine Models of the Human Amyloidoses

Amyloidosis A (AA)

In humans AA amyloidosis occurs as a consequence of chronic inflammation, infectious or non-infectious. It is found in approximately 5% of patients with rheumatoid arthritis, an unknown proportion of individuals with chronic infections and a significant proportion of non-Ashkenzi Jews with Familial Mediterranean Fever and individuals with other genetic abnormalities leading to autoinflammatory disorders related to the innate immune system [8].

With respect to the human systemic disorders, the inflammation inducible murine AA model in which susceptible strains of mice were exposed to a variety of inflammatory stimuli (subcutaneous injections of casein, Freund's adjuvant, turpentine, silver nitrate) had been studied long before recombinant proteins became available [9]. The isolated fibril was the first non-immunoglobulin characterized chemically, thus named Amyloid "A" [10]. The model

provided insight regarding the nature of the precursor, i.e. the serum acute phase apolipoprotein SAA, and the role of inflammatory cytokines (IL6, IL1 and TNFα) in stimulating SAA production via NfκB, with the subsequent generation of AA as the direct fibril forming precursor by proteolysis of SAA, apparently by macrophage proteases [11-14]. The model suggested that genetically controlled differences in precursor structure could determine individual susceptibility to amyloid deposition in response to a chronic inflammatory stimulus. It also served as a test system for molecules designed to inhibit tissue deposition by interfering with the interaction between AA aggregates and matrix proteins that were subsequently evaluated in human clinical trials [15;16].

The murine AA model has also raised the issue of whether “infectivity” plays a role in other forms of amyloidosis in addition to the prionoses. It has been shown in many laboratories that injection of sonicated splenic extracts from mice with AA deposition, when given in conjunction with an inflammatory stimulus, will accelerate the appearance of amyloid fibrils in a naïve recipient from weeks to days [17]. The active component (originally known as amyloid enhancing factor or AEF), which appears to serve as a seed, is the growing end of the fragmented fibrils. Amyloid A was the first form of systemic amyloidosis to display the seeding phenomenon *in vivo.* Tissue deposition can also be accelerated by the injection of fibril fragments from a variety of sources including fragmented fibrils formed from transthyretin peptides, silk, and bacterial curlin protein as well as AA fibrils themselves [18]. It has also been established that oral ingestion of such seeds may serve as accelerants [19], [20]. In principle this is felt to differ from the infectivity of the true prion disorders, since in the case of prions, the animals would not get aggregation and deposition if they did not see the scrapie conformer or a similar seed. Hence, there may be a meaningful distinction between initiation (as seen in the prionoses) and acceleration as seen with AEF and murine ApoAII (see below).

Transgenic technology has not played a major role in the investigation of AA disease. However, three sets of transgenic studies have been informative in the investigation of AA pathogenesis. Recently it has been shown that, as expected, IL-6 transgenics will develop AA amyloid deposition on the basis of increased SAA synthesis and processing and such animals can be utilized in testing for factors which accelerate the process [21].

In a second transgenic study over-expression of the gene encoding heparanase revealed that shortening the length of the heparan sulfate chains in matrix proteoglycans made animals resistant to AA deposition [22]. In other AA related studies gene silencing has been utilized to investigate the role of serum amyloid P-component (SAP), the murine acute phase protein found in all amyloid deposits, in the pathogenesis of AA amyloidosis in mice. Two independent studies of separately derived strains in which its gene (*APCs*) was silenced by targeted disruption, showed that inflammation-induced AA occurred at a moderately decreased rate relative to controls, and that the rate of absorption of the deposits after stopping the stimulus was no different in the SAP knockouts and controls. Thus, despite being present in the deposits of all forms of amyloid, SAP was not required for either fibril deposition or stability *in vivo* [23;24]. One can envision experiments in which a gene or genes encoding the protease(s) required for the processing of the SAA precursor to AA might be silenced with a profound effect on the deposition phenotype. However, such experiments have not been reported.

Apolipoprotein AII

A second naturally occurring animal amyloidosis was described by Japanese investigators who, in the course of breeding sub-strains of AKR mice to select for longevity, discovered that several of their variant strains uniformly developed amyloid deposition late in life [25]. This was not surprising since the increasing frequency of amyloid deposition with age had been previously noted in human autopsies [26]. The amyloid consisted of intact apolipoprotein AII [27]. Mouse strains bearing the amino acid glutamine at position 5 (ApoA2^{c}) were found to be

susceptible to this form of amyloidosis, while strains having proline in the same position (ApoA2^{a} or 2^{b}) were not [28]. The potential for amyloidogenesis could be transferred to a non-amyloid forming strain by making it congenic for the amyloidogenic isoform [29]. While AA deposition was also found with aging in some strains of mice and some mice had both forms, there did not appear to be cross seeding in the naturally occurring diseases. However subsequent experiments in which fibrils or fibril fragments were injected into animals representing each model suggested either an additive or reciprocal relationship in the two forms of fibril deposition [30]. These results, while interesting and suggesting that cross seeding can be induced experimentally, are not consistent with cross-seeding in the natural course of either AA or ApoAII deposition in mice. As in the AA model, injection or feeding of ApoAII fibril fragments could accelerate deposition [31]. The ApoAII amyloid could also be transmitted by fecal ingestion or vertically during nursing [32]. Hence the phenomenology of acceleration by seeds was similar to that seen in the AA model.

Many, but not all, amyloid precursors form fibrils in a process of nucleated polymerization [33]. Addition of small amounts of fibril fragments (seeds) to the *in vitro* reaction accelerates the process of fibrillogenesis [34]. It has been assumed that the association of various forms of amyloidosis with aging reflects a latent period during which the seeds form. The seeding experiments in the AA and ApoAII models do not necessarily support this argument. In both these systems the precursor (SAA or ApoAII) is present in very high concentrations either as the result of inflammation (SAA) or in response to diet ApoAII [35]. Both precursor molecules are apo-lipoproteins, hence have a sufficient degree of hydrophobicity to be potentially unstable in aqueous media. Both are amyloidogenic when freed from their HDL ligand. SAA requires cleavage to generate the structure that has optimal fibril forming capacity. Inflammatory cells provide both a cellular source of appropriate protease activity as well as the amyloidogenic substrate. These appear to be special cases, peculiar (but not exclusive) to the mouse. The relatively high frequency of these conditions in particular mouse strains and their relative rarity in humans may make them less informative as models of human disease than interesting experimental systems in their own right.

Prion Diseases

The same can be said of the prionoses. Although the apparent epidemic of variant Jakob-Creuzfeldt disease associated with the ingestion of beef from animals with Bovine Spongioform Encephalopathy (BSE or “mad cow disease”) and its genetic counterpart (CJD) indicate that there is a human homolog [36]. Here again much of the characterization of murine and hamster scrapie was achieved well before the availability of transgenic animals. The notion of “protein only infectivity” mediated by templated misfolding of a native protein was a novel idea, which appears to be generally correct [37]. Exposure via ingestion followed by transport to the brain through lymphatics and a long incubation period is well established although we are still far from full understanding of the process. The demonstration of strain differences related to specific protein structures and the variability of such structures in a given strain is under active investigation as is the basis for strain differences in pathogenetic effects, i.e. more amyloid fibril formation in some strains and more spongioform change in others [38]. As in many of the pathogenic amyloidoses there is accumulating evidence that the mature fibrils are not the toxic element rather it is oligomeric pre-fibrillar species that are the major pathogenetic species. These data are quite convincing in tissue culture but *in vivo* confirmation remains an investigative goal.

Transgenic/knockout animals enabled the performance of the critical experiments demonstrating that host specificity was a function of the endogenous PrP^{c} gene since murine PrP^{sc} was not pathogenic in mice in the absence of a functional PrP^{c} gene [39]. Similarly the demonstration that BSE aggregates could affect mice in which the endogenous PrP^{c} had been

replaced by its human homolog was the best laboratory evidence for transmission of that agent into humans [36].

AL Amyloidosis

Transgenesis technology and its gene silencing variant have allowed the development of a number of potentially relevant models of human protein misfolding diseases but have not been fruitful for some human amyloidoses. For instance there have been numerous attempts to generate a transgenic model of the systemic amyloidosis related to immunoglobulin light chain deposition (AL). No successful models have been reported, nor has the reason for the lack of success been defined, although it may be that the amyloidogenic potential of a particular light chain structure can only be realized when the protein is synthesized and secreted to a level equal to or greater than the critical concentration required for its aggregation under physiologic conditions. *In vivo* this may require a degree of clonal expansion that has not yet been achieved experimentally.

Transfection of the murine hybridoma parent cell line SP2 with an amyloidogenic human light chain and injection of the cells into a recipient animal was not experimentally useful since the SP2 cells grew too quickly to allow any analysis of the process of amyloid formation or deposition even if it had occurred. The only model, which has had some success, is highly artificial and involves injecting large amounts of an amyloidogenic precursor light chain either intraperitoneally or under the skin [40]. A mass forms consisting largely of fibrillar material surrounded by macrophages and neutrophils which is ultimately resorbed. The host response to the mass suggested that an antibody directed toward epitopes available on the aggregates might enhance the inflammatory response and hasten clearing. This is a cumbersome model that has little resemblance to the development of the disease *in vivo*. Nonetheless anti-amyloid antibodies have been generated, shown to accelerate clearing of the deposits and have been humanized for use in clinical trials [41].

Transgenic Considerations

What kind of information would emerge from transgenic disease models that might be more difficult or impossible to obtain in other experimental systems? Since the extracellular amyloidoses are systemic, with deposition frequently taking place at sites distant from the site of synthesis an organismal rather than tissue culture approach would seem to be favored. Models should allow the development of more precise notions of pathogenesis and the roles of proteins other than the actual precursor in facilitating or inhibiting amyloid generation and deposition. In the most sophisticated instances, gene replacement (knock in) technology allows the replacement of a precursor molecule with a gene or exon encoding an amyloidogenic sequence that differs from the original. This approach has been utilized to demonstrate the requirement for Cys10 in the TTR V30M structure in order to achieve aggregation and deposition in TTR V30M transgenic animals [42]. Such experiments validate data obtained *in vitro* on the effects of specific structural changes on the process of fibril formation.

The effects of genetic factors, not readily apparent in the human diseases, *in vitro* or in tissue culture settings, can be assessed in transgenic animals, particularly in crosses in which genes encoding potentially interactive proteins are silenced (see above re:SAP knockouts). Apolipoprotein E knockouts have been informative, although not consistent, in studies of pathogenesis in Alzheimer's disease (AD) models [43]. Animal models lend themselves to testing the roles of diet and other environmental factors on disease development in the presence of a *bona fide* precursor transgene. The most obvious use is in the evaluation of therapies using both small molecule and biologic approaches to prevention and treatment.

What properties would we like in such models? Ideally one would like the precursor to be driven by its endogenous promoter so that its tissue expression reflects what occurs *in vivo* with respect to known physiologic and environmental influences (so-called "construct validity"). It would be preferable to have the gene expressed at levels similar to those seen *in vivo*, usually achieved by integration of a single functional gene copy or the diploid state in a homozygous cross. This becomes particularly important in models in which a therapeutic is designed to bind to the precursor with a defined stoichiometry. Multiple copy number models might require much more drug for effect than would be necessary in patients in whom there may be only one or at most two genes encoding the amyloidogenic protein. Thus an agent that might be effective in patients might be ineffective or toxic in a model that depended on overproduction of the amyloid precursor to obtain the disease phenotype simply because there is too much precursor (lack of "predictive validity").

One would also like the model to display both clinical and pathologic phenotypes that resemble those seen in human patients with the disease being modeled, including its temporal pattern of development, i.e. more disease with increasing age in the case of the age dependent amyloidoses and tissue distribution of deposits ("face validity"). Once it was established that the model reflected the human disorder one would like to be able to accelerate its development (without changing the responsible molecular events) so data could be acquired in a more compressed time frame.

For some models, while mice offer the advantage of extensive knowledge of their genetics, their small size makes certain types of manipulations and studies difficult. Some laboratories have chosen to develop transgenic rat models for conditions in which these issues (e.g. organ transplantation therapies) are serious considerations [44]. In other circumstances lentiviral or adenovirus associated viral vectors encoding amyloidogenic alleles have been injected intracerebrally under stereotactic control to target specific brain regions. These methods are easier in rats but have now been adapted to mouse studies as well. In addition the larger rat brains allow more informative use of imaging technology.

For impatient investigators the introduction of amyloid precursors into worms (*c. elegans*) and flies (*d. melanogaster*) compresses the time necessary to obtain a disease phenotype [45;46]. However the relationship between that cellular and molecular phenotype and human disease may be problematic. On the other hand the comparability of these post-mitiotic model systems to mammalian post-mitotic tissues, such as neurons or myocytes, suggests that the molecular aspects of some of the models may be extremely useful in the analysis of those disorders in more long-lived species. This has been evident in a series of experiments in worms transgenic for a gene encoding a form of the Huntington disease protein with a poly-Q region containing a stretch of glutamines that does not normally aggregate. By silencing genes encoding various components presumed to be involved in the handling of misfolded proteins the threshold for aggregation is lowered and the pathologic phenotype appears [47]. While this may not be an accurate "disease model" it allows detailed investigation of cellular and molecular pathogenesis and could be used as an *in vivo* screen for inhibitors that might be useful in the analogous human disorders.

At this moment none of the murine transgenic models of any of the systemic amyloidoses exhibit ideal characteristics. While some of the variation reflects general differences between humans and mice (the details of which are not fully understood), some are a consequence of particular features of murine molecules and their interaction with transgene products [48].

The Transthyretin (TTR) Amyloidoses

There are three human disorders characterized by the extracellular deposition of TTR aggregates, Familial Amyloidotic Polyneuropathy (FAP), Familial Amyloidotic

Cardiomyopathy (FAC) and Senile Systemic Amyloidosis (SSA). The first two are caused by mutations. The last is the result of age dependent cardiac deposition of the wild type protein. In each case the homo-tetramer releases a monomer subunit, which misfolds and is subject to aggregation and tissue deposition in its target organ [49]. What determines the site of deposition of any of these proteins is still unknown.

The original TTR transgenics utilized cDNA's encoding the amyloidogenic allele V30M TTR driven by the relatively promiscuous metallothionein promoter. The animals showed early deposition in all tissues in which the mRNA was identified, however in tissues other than the liver and choroid plexus there was no expression of the endogenous *TTR* gene, suggesting that such a construct would not generate a useful disease model since it was being expressed in tissues not normally synthesizing the protein [50]. The animals did not develop peripheral neuropathy, the hallmark of the human disease. Subsequently the intact human gene with most of its known regulatory sequences was reconstructed from two partial genomic clones isolated from a human EcoR1 library made from an FAP patient and used to generate several additional transgenic strains [51]. One of these contained 6.0kb of sequence upstream of the initiation site, which showed appropriate tissue specific expression and tissue deposition (gut, heart, skin, kidney) beginning at about 1 year of age. These animals contained 30-60 copies of the human gene, but still did not display peripheral or autonomic neuropathy. Nonetheless they were considered moderately successful.

The role of environment had to be addressed when these transgenics were moved from a conventional animal facility to a specific pathogen free facility. With that move the mice stopped developing tissue deposits to the same extent as previously [52]. As expected they showed a difference in the gastrointestinal flora in the two locales and hypothesized that some degree of inflammation was required to stimulate the formation of tissue aggregates [53]. On the surface this explanation seems unlikely since hepatic TTR synthesis, which is responsible for producing the bulk of circulating TTR, the fibril precursor, is down-regulated in the course of inflammation by the action of inflammatory cytokines [54]. It is also not clear whether genetic drift has been completely eliminated as a proximal or contributory cause. Other TTR transgenics in other laboratories have yielded perfectly valid phenotypes when reared under SPF conditions.

Transgenics made from the wild type and mutant TTR genes of an individual carrying the highly unstable L55P TTR mutation provided several interesting insights into the nature of transgenic animal models. Both genes used to produce the transgenics were cloned as 19.2 kb genomic fragments containing all the known regulatory sequences of the normal TTR gene [55]. Animals bearing the wild type gene contained 100 copies and displayed amyloid deposition in the kidneys (80%) and the hearts (50%) after 18 months. Both strains showed tissue expression in the pattern expected from studies in humans, i.e. liver, choroid plexus, eye, gut and kidney. In the animals bearing the wild type gene, males showed greater deposition than the females. In both sexes younger animals showed non-amyloid (diffuse) deposits in the same organs at an earlier age than the Congophilic deposits. The occurrence of fibrillar deposits seems to be both a function of TTR concentration and age (duration of exposure) a phenomenon that was subsequently confirmed in the human disease when non-fibrillar TTR deposits were found in the peripheral nerves of Portuguese patients with early FAP [56].

The age and gender dependent deposition of the wild type protein resembles that seen in the human SSA disorder, Transcription analyses of these animals indicated that cardiac deposition occurred when the liver did not respond to the presence of the many copies of wild type TTR with an unfolded protein response (UPR) and subsequent transcription of chaperone genes and elements of the ubiquitin-proteasome system (Buxbaum et al unpublished). In animals with such a transcriptional response histologic cardiac deposition did not occur and no oxidative

stress response was seen in the hearts. On the other hand, livers from animals with cardiac deposits showed no UPR or chaperone response while their hearts showed an exuberant transcriptionally mediated oxidative stress response. At this time it is unknown whether the tissue responses will be the same to the normal concentration of a thermodynamically unstable TTR as they are to large amounts of the wild type protein. If the absolute amounts of misfolded TTR are equivalent, representing a relatively large proportion of the total amount of the unstable protein and a small proportion of the wild type molecule, the processes should be similar. This remains to be proven experimentally.

The strain derived from the mutant L55P TTR gene contained a single copy of the gene and showed no deposition phenotype even at 2 years of age in animals homozygous for the human construct. Inoculation of these animals with fibrils made from TTR peptides that behaved as active AEF in the inflammation induced AA model did not result in the development of TTR deposits in these mice [57;58]. In subsequent studies the mutation was crossed onto the murine TTR knockout background. One-third of those animals showed deposition suggesting that the murine TTR molecule inhibited the aggregation and deposition of the highly unstable human molecule [59]. Combined *in vivo* and *in vitro* studies subsequently showed that in the presence of the murine TTR gene, highly stable human-mouse hetero-tetramers were formed, thus reducing the availability of the free human monomer precursor of the aggregates [48]. Further experiments showed that administration of diflunisal, a molecule that binds in the thyroxine binding pocket of TTR and is currently in clinical trials in patients with FAP, stabilized the human L55P molecule to denaturation by urea to the same extent as did the murine protein in a fashion similar to that seen when Portuguese V30M TTR carriers also bear the T119M TTR allele in trans [60].

Other investigators have used the L55P allele in different molecular settings to accelerate pathogenesis. When the animals were crossed with HSF1 knockout mice that were unable to mount a chaperone response, the offspring showed relatively early gastrointestinal deposits and, for the first time, deposits in sciatic nerve and dorsal root ganglia [61]. The demonstration that silencing a critical transcriptional regulator of the unfolded protein response accelerated disease confirmed its involvement in protection against tissue damage produced by TTR aggregates. What is not clear from these studies is whether the absence of HSF1 is making target cells more sensitive to toxicity of extracellular aggregates, whether its impact is on the liver's capacity to adequately chaperone misfolded TTR synthesized in the hepatocyte or whether TTR is being made in dorsal root ganglia and the absence of HSF1 results in cell death based on aggregation of locally synthesized TTR?

A transgenic strain has also been constructed using the gene encoding I84S TTR, a very interesting mutation in which the TTR retinol binding protein binding site is disrupted. Neither amyloid nor diffuse TTR deposits have been seen in these animals over many generations, even when crossed onto the murine TTR knockout background [62]. These animals appear to carry 8-10 copies of the human gene. They have been used as a model system in which to test the efficacy of TTR anti-sense to specifically silence transcription of the mutant allele [63]. Since these animals have no deposition phenotype the effect of such silencing on deposition cannot be judged. Successful silencing would provide proof of principle of the approach to show that production of the protein can be diminished in the liver.

Thus it is quite clear that murine transgenic models of the TTR amyloidoses have provided interesting insights into the pathogenesis of the human disorders which they were generated to model. However, the necessity for over-expression to overcome the stability of murine-human hetero-tetramers and the apparent differential processing of misfolded-prone proteins between humans and mice or the need to cross the animals onto mice that have deletions in genes with major homeostatic functions other than simply chaperoning the amyloid precursor,

have made them less than optimal tools for either fine analysis or experimental therapies. There have been recent successful efforts to “knock in” human TTR transgenes into the murine TTR locus [64]. It remains to be seen whether experiments with these strains can overcome the experimental disadvantages of the earlier models.

Islet Amyloid Polypeptide (Amylin, IAPP)

Homogeneous eosinophilic deposits were first described in the pancreas at autopsy of diabetic humans in 1901. Their amyloid nature and association with aging in diabetics taking insulin was established in autopsy series much later and the identification of the fibril as IAPP or amylin almost simultaneously by two laboratories in the mid 1980's [65];[66]. This is a localized form of amyloid with systemic effects thus is probably best studied in an organismal context. Comparative structural studies showed that the amino acid residues 20-29 were critical for the amyloidogenicity of the 37 amino acid mature peptide. Proline at position 28, the residue found in mouse, rat and hamster, was anti-amyloidogenic [67]. This observation prompted several laboratories to create transgenic mouse lines, correctly assuming that there would be no endogenous IAPP amyloidogenesis to confuse the interpretation of the data.

Since it had previously been established that insulin and IAPP were co-expressed and secreted by the pancreatic β-cell each laboratory used some form of the rat insulin promoter to drive human IAPP cDNA [68-71]. In some laboratories intron fillers derived from either globin or albumin were used with the purpose of enhancing functionality of the transgene. The number of integrated copies of the human construct was not specifically noted in any of the reports. Strain backgrounds varied with FVB/N, C57Bl/6 and C57Bl/6 x DBA/2 all being used as hosts, with subsequent crosses onto a variety of backgrounds to determine the effects of other genes (e.g. ApoE, Agouti, Ob/Ob)[72;73]. The endpoints for all were increased IAPP concentrations in the serum, human IAPP staining in β-cells, pancreatic amyloid deposition with fibrils derived from human IAPP and hyperglycemia or overt diabetes. Beta cell IAPP synthesis was shown in some of the transgenic animals in virtually all the experiments. Increased serum IAPP was also frequent. However in only one model did pancreatic amyloid develop without further experimental manipulation [70]. It was ultimately established that procedures that increased insulin resistance (glucocorticoids, high fat diets, mating with obesity predisposed strains) all increased the frequency of pancreatic amyloid deposition, β-cell loss and hyperglycemia [74; 75]. More refined analyses have suggested that non-fibrillar aggregates were cytotoxic, in some models inducing β-cell loss prior to significant formation of mature amyloid deposits [76].

As a disease model these animals have validated the notion of IAPP amyloidosis as playing a role in the pathogenesis of type II diabetes. While relative or absolute insulin insufficiency may be primary the subsequent insulin resistance stimulates the co-expression of the IAPP and insulin genes. In these models the magnitude and cell site of that expression was insured by driving the IAPP construct with an insulin promoter. Since it has been shown *in vitro* that insulin as well as rat and presumably mouse IAPP inhibit human IAPP fibril formation (chaperone) perhaps it is the relative amount of each set of molecules that determines the rate and extent of oligomerization and fibril formation and accounts for the variability among the different transgenic strains. One of the variables in disease development might be the presence of mixed human/rodent IAPP aggregates that are less fibrillogenic than homo-IAPP complexes in a fashion similar to that seen with human/murine TTR hetero-tetramers. These models continue to be explored with the notion that stabilizing IAPP will reduce the rate of β-cell loss and make molecules that enhance insulin production and release or reduce peripheral insulin resistance more effective, thus increasing the effectiveness of treatment.

Gelsolin

Gelsolin is the precursor protein in the Finnish form of Familial Amyloidotic Polyneuropathy, which is associated with skin, facial nerve, cardiac and renal amyloid deposition [77]. It represents another variation on the theme of systemic amyloidosis. In this instance synthesis and deposition of the precursor occurs at multiple sites. In the human disorder a mutation at position D187 to N or Y (D187N/Y) in plasma gelsolin compromises Ca2+-binding in domain 2 enabling aberrant cleavage by furin generating a secreted 68 kDa fragment (C68) that can be further cleaved in the extracellular space by a type I matrix metalloprotease, such as MT1-MMP, to generate the major (8 kDa) and minor (5 kDa) amyloidogenic fragments that deposit in the tissues [78]. A single transgenic strain has been reported in which the mutant gelsolin has been expressed in C57Bl/6 mice under the control of a creatine kinase promoter in the context of a 5′ globin intron and a bovine growth hormone poly A site (Page L, et al PNAS (USA)(in press).

As expected, because of the promoter choice, gelsolin synthesis was seen only in cardiac and skeletal muscle. Congophilic gelsolin deposits were found in capillaries and the skeletal muscle endomysium. Deposition increased with age. The model appears to faithfully reproduce the molecular events resulting in the same amyloidogenic fragments seen in the human disease but results in both extra and intracellular aggregates rather than only the extracellular deposits described in human gelsolin amyloidosis. Pathologically vascular disease is less prominent and the muscle lesions of the animals appeared to be more like inclusion body myositis, a sporadic muscle disease of the elderly in which a variety of fibrillogenic proteins have been found to be deposited, the most prominently described of which has been Aβ [79].

Alpha-1-Anti-trypsin (AAT) (Z-variant) Deficiency

While not originally recognized as a protein misfolding disorder and certainly not having any of the characteristics of the systemic amyloidoses, i.e. Congophilic extracellular deposition at sites distant from the site of synthesis, homozygous AAT deficiency has the characteristics of both an LOF and GOF mutation [80]. The affecteds have diminished levels of a specific circulating serpin (serine protease inhibitor), exhibit recurrent pulmonary disease and ultimate respiratory failure because of excessive neutrophil elastase activity in the lungs causing destruction of lung tissue. It is an LOF disorder and belongs to the class of serpinopathies.

The mechanism of aggregation in the serpinopathies is quite different from that in the amyloidoses. The effect of the mutation is to interfere with the generation of the active site in which the target protease must fit allowing insertion of the domain of another AAT molecule to form a dimer with subsequent homo-polymer propagation. A similar mechanism has been proposed for neuroserpin in the dementia familial encephalopathy with inclusion bodies (see below).

The Z variant is one of the few examples of intracellular aggregation of a protein normally secreted by hepatocytes, since the liver cell seems to be particularly well-suited to deal with potentially misfolded proteins, e.g. TTR mutants. The AAT liver displays intracellular inclusions, hepatocyte loss, fibrosis and an increased frequency of hepatocarcinoma. All of these features have been recapitulated in the transgenic models of the disease [81;82]. Further it has been possible to treat these animals with small molecules that behave like molecular chaperones [83].

Neurodegenerative Protein Misfolding Diseases

We have characterized the neurodegenerative protein misfolding diseases as extrinsic, i.e. the prionoses, or intrinsic, i.e. independent of external agents. The latter constitute the majority of the described disorders. In these diseases the precursor is synthesized and misfolds either within

or in proximity to the target of its toxicity. Conceptually these disorders should be readily approachable using tissue culture techniques rather than requiring animal models, since all the action is taking place in a confined space. The utility of animal models relates to two features of most of these disorders. To some extent they are all age dependent, a quality difficult to establish in tissue culture. Further, it is desirable to ultimately validate any molecular observation with a behavioral readout. Thus a variety of animal models of Alzheimer's disease, Parkinson's disease, Huntington's disease, Tauopathies, ALS have been generated and extensively employed in a variety of settings and have been the subjects of many excellent reviews [84-88;88;89].

Alzheimer's disease (AD)

In many ways the attempts to make useful murine transgenic models of human AD although imperfect, have been among the most informative. Over the last several years there have been excellent detailed reviews of these models to which the reader can refer for specific information [90-93]. I will not review them here except to draw general lessons, some of which have been discussed with respect to the systemic amyloidoses in earlier sections.

Lesson number 1—mice are not humans. We should not expect the expression of a human disease-associated gene in a mouse to precisely duplicate the human disease of interest. For instance, while Tau phosphorylation has been seen in some transgenics bearing either a mutant Aβ gene or a combination of mutant Aβ and mutant presenilin 1 (PS-1) genes, neurofibrillary tangles (a characteristic feature of human AD) are only seen consistently in the triple transgenics that also carry a Tau mutation [94-96]. Few studies have been directed at examining differences between mice and humans with respect to defining systematic guidelines applicable to all or most transgenic models.

Lesson number 2—Phenotypes produced by insertion of a transgene encoding a gain of function (GOF) protein are quantitatively much more dose-dependent than models in which a gene deletion was generated to reflect a loss of function (LOF) disorder. While homozygous gene deletions may be embryonic lethal and hemizygotes give a survival disease phenotype in “LOF” models, the number of copies of a transgene encoding a GOF protein can determine whether the disease phenotype is penetrant in the model, its temporal development and its severity, since in general the level of protein production parallels gene copy number. It is not clear why over-expression is required in many or all of these models. It is possible that murine cells, particularly neurons, are better equipped to chaperone or dispose of misfolded proteins than human cells of similar lineages, although there are no quantitative data addressing this question.

Lesson number 3—Genetic background makes a difference. The same mutation carried in different mouse strains may show substantial variation in phenotype, from no disease to early death in some cases [97]. This should not be surprising since humans are not inbred (for the most part) and could be used to an advantage in understanding the pathology by mapping the responsible contributory loci. The corollary from human genetics is that “there are no single gene diseases”. For instance Familial Amyloidotic Polyneuropathy (FAP) caused by the Val30Met transthyretin (V30M TTR) mutation behaves as an autosomal dominant disease (see above). Yet the penetrance is similar in Japanese and Portuguese carriers but far less so in Swedes with the same mutation [98]. Even within the Portuguese population the age of onset of disease can vary dependent on which parent contributes the mutant allele as well as genetic variation at other loci that may be involved in pathogenesis [99;100].

Lesson number 4—Environment matters. Variation in phenotype from laboratory to laboratory reflecting differences in diet, husbandry, maintenance and factors that have not yet

been elucidated can all impact on disease expression and the potential validity of the model [52];[101];[102].

Lastly is the element of publication bias, i.e. the published papers are much more likely to document successfully generated transgenic strains than those in which no phenotype was obtained.

Cystatin C (Cerebral Amyloid Angiopathy or Hereditary Cerebral Hemorrhage with Amyloidosis, Icelandic Type (HCHWA-II)

Many of the human amyloidoses affect blood vessels. In this condition a mutation in Cystatin C, a cysteine protease inhibitor, Leu68Gln, causes it to dimerize by a process thought to involve domain swapping followed by aggregation within the walls of cerebral blood vessels [103]. The aggregation is associated with multiple cerebral hemorrhages in the carriers. However, mice made transgenic for the human mutant allele with significant over-expression did not display Congophilic angiopathy [104].

Immunohistochemical analysis of AD brains has shown the presence of cystatin C in both plaques and cerebral vessels. When wild type cystatin mice were crossed with the APP23 AD model mice there was a 50% reduction in cortical plaques, a 50% reduction in $A\beta_{1\text{-}40}$ and a 25% reduction in $A\beta_{1\text{-}42}$ concentrations in the brain at age 14.5 months. Similar effects were seen when the cystatin C animals were crossed with the TG2576 AD model mice. These experiments are consistent with *in vitro* experiments showing direct binding of cystatin C to $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ with inhibition of fibril formation [104].

BRI2 Related Dementias

Mutations in the *BRI2* gene have been identified in familial British Demential and familial Danish dementia [105;106]. A point mutation (FBD) and a decamer duplication (FDD) allow read through the normal stop codon to yield a protein 11 amino acids longer than in the native type II transmembrane protein. Furin type proteolysis generates a 34 amino acid fibril precursor which forms Congophilic vascular deposits (Congophilic angiopathy) “soft” parenchymal plaques and fibrillar deposits in the brains of the carriers. Attempts to generate a transgenic model for FBD were not successful [107]. However transgenic animals produced by using a cDNA enoding the Danish BRI2 extended sequence driven by the murine prion protein promoter injected into C3HeB/FeJ oocytes, subsequently bred into C57Bl/6 for 10 generations, developed Congophilic angiopathy at 7 months of age. Punctate and diffuse antibody positive deposits and gliosis were seen in the regions of the deposits [108].

When Adenovirus Associated Viral vectors containing sequences encoding the Bri2 protein were injected intracerebrally into AD model mice the neuropathologic features of AD were suppressed [109]. These studies were consistent with in vitro experiments showing that BRI2 bound to amyloid β precursor protein interfering with its processing by secretases [110;111], Hence like cystatin C, the wild type form of the amyloidogenic precursor interferes with molecular events involved in AD pathogenesis.

Familial Encephalopathy with Neuroserpin Inclusion Bodies (FENIB)

In 1996 a kindred was reported with dementia beginning in their 40's, apparent autosomal dominant inheritance and the striking appearance of neuronal inclusion bodies in the cortex and elsewhere [112]. The inclusions were unique (Collins bodies) and were found to be composed of aggregates of the serine protease tissue plasminogen activator inhibitor neuroserpin. Mutations in the gene were identified in independent kindreds (P112, S52R, S49P). The wild type gene was subsequently identified immunohistochemically in AD plaques.

In vitro incubation of the wild type protein with amyloidogenic Aβ peptides inhibited fibril formation and their cytotoxic effect on PC 12 cells [113].

Transgenic flies were generated using the wild type gene [113]. Interestingly these flies were embryonic lethal when expressed throughout the larvae and generated a mutant eye phenotype when controlled by the $GAL4^{GMR}$ promoter. When the flies were crossed with flies transgenic for $A\beta_{1-42}$ the embryonic lethal phenotype was abolished, indicating that the Aβ interaction inhibited the protease inhibitory activity of the serpin molecule. Although the *in vitro* experiments indicated that Aβ fibril formation was inhibited by the interaction, effects on the Aβ phenotype of the flies were not reported.

Gelsolin and Transthyretin and AD

The apparently inhibitory interactions of wild type Cystatin, BRI2 and Neuroserpin with Aβ to reduce fibril formation are interesting since mutants of each of these proteins are fibril or aggregate precursors *in vivo* and all have been found associated with plaques in AD brains. Studies have also been reported in which animals transgenic for wild type gelsolin, apparently expressed only in the liver (after hydrodynamic injection of a wild type gelsolin cDNA) reduced cerebral Aβ burden, a phenomenon attributed to the peripherally synthesized gelsolin behaving as a “plasma sink” for potentially amyloidogenic Aβ peptides [114]. A gelsolin-Aβ interaction has also been demonstrated *in vitro* [115]. These studies require further confirmation.

Analysis of the interaction between wild type transthyretin and Aβ *in vitro* have shown interaction between the two proteins as well as inhibition of fibril formation independently in several laboratories [116;117]. APP23 AD model animals transgenic for TTR show suppression of both the neuropathologic and behavioral phenotypes, while silencing of the endogenous TTR gene accelerated the development of the characteristic neuropathologic findings in two different murine AD models [118;119].

Do these interactions *in vitro* merely reflect the legendary stickiness of the $A\beta_{1-40}$ and $A\beta_{1-42}$ peptides or are they biologically meaningful? Perhaps they indicate that wild type proteins that are predisposed to become amyloidogenic in the presence of a structure-perturbing amino acid substitution share a set of physical properties that on the one hand predispose to aggregation, but under relatively physiologic conditions are capable of reacting with similar proteins in a manner that allows both to assume (or re-achieve) native stable conformations. Are the effects in the transgenic models only properties of these relatively artificial systems or do they reflect *in vivo* interactions that play a role in the pathogenesis of human AD? Obviously these observations could not have been made in the absence of animal models of AD. In order to fully establish these phenomena as relevant for these and other proteins it would seem that an *in vitro* interaction must be observed with recombinant forms of the proteins with reasonable stoichiometry. Inhibition of fibril formation should be clear. Expression of a transgene encoding the putatively interacting protein should impact on the pathology and physiology of the model disease and silencing of the endogenous gene should have the opposite effect on the disease phenotype. All should be confirmed in more than one laboratory. Lastly, or perhaps firstly, there should be some evidence that there is a relationship of the protein in question to the human disease. To date this has only been achieved for the interaction between TTR and Aβ.

Coda: Are the models worth the time and trouble?

Each animal model is akin to a single patient. To the extent that we can examine the organismal effects of what may be a single mutation in a single protein they are useful. Whether they can provide the same mechanistic insights as cultured cells of the appropriate lineage is less certain for many of the disorders. They certainly afford the opportunity to examine gene-gene

interactions and allow the detection of effects at a distance. They raise mechanistic questions that may ultimately only be answered in more reductionist experimental systems. Given those attributes the investigator must decide whether the transgenic or other animal model approaches are suitable to address their particular biologic problem.

References

1. Virchow R. Über den Gang der amyloiden Degeneration. Virchows Arch 1855;8:364–368.
2. Kisilevsky R. The relation of proteoglycans, serum amyloid P and apo E to amyloidosis current status, 2000. Amyloid 2000;7:23–25. [PubMed: 10842701]
3. Wilhelmus MM, de Waal RM, Verbeek MM. Heat shock proteins and amateur chaperones in amyloid-Beta accumulation and clearance in Alzheimer's disease. Molec Neurobiol 2007;35:203–216. [PubMed: 17917109]
4. Glenner GG. Amyloid deposits and amyloidosis. The β-fibrilloses (first of two parts). N Engl J Med 1980;302:1283–1292. [PubMed: 6154243]
5. Glenner GG. Amyloid deposits and amyloidosis. The β-fibrilloses (second of two parts). N Engl J Med 1980;302:1333–1343. [PubMed: 6990257]
6. Conway KA, Harper JD, Lansbury PT. Accelerated *in vitro* fibril formation by a mutant alpha synuclein linked to early-onset Parkinson's Disease. Nat Med 1998;4:1318–1320. [PubMed: 9809558]
7. Johnson KH, Westermark P, Sletten K, O'Brien TD. Amyloid proteins and amyloidosis in domestic animals. Amyloid 1996;3:270–289.
8. Buxbaum J. The genetics of the amyloidoses: interactions with immunity and inflammation. Genes Immun 2006;7:439–449. [PubMed: 16837932]
9. McAdam KPWJ, Sipe JD. Murine model for human secondary amyloidosis: genetic variability of the acute phase serum protein SAA response to endotoxin and casein. J Exp Med 1976;144:1121–1127. [PubMed: 978136]
10. Eriksen N, Ericsson LH, Pearsall N, Lagunoff D, Benditt EP. Mouse amyloid protein AA: Homology with nonimmunoglobulin protein of human and monkey amyloid substance. Proc Natl Acad Sci U S A 1976;73:964–967. [PubMed: 815910]
11. Rokita H, Shirahama T, Cohen AS, Sipe JD. Serum amyloid A gene expression and AA amyloid formation in A/J and SJL/J mice. Br J Exp Pathol 1989;70:327–335. [PubMed: 2765389]
12. Westermark GT, Engström U, Westermark P. The N-terminal segment of protein AA determines its fibrillogenic property. Biochem Biophys Res Commun 1992;182:27–33. [PubMed: 1731787]
13. Baumann H, Gauldie J. Regulation of hepatic acute phase protein genes by hepatocyte stimulating factors and other mediators of inflammation. Mol Biol Med 1990;7:147–155. [PubMed: 1692952]
14. Kluve-Beckerman B, Manaloor JJ, Liepnieks JJ. A pulse-chase study tracking the conversion of macrophage-endocytosed serum amyloid A into extracellular amyloid. Arthritis Rheum 2002;46:1905–1913. [PubMed: 12124875]
15. Kisilevsky R, Lemieux LJ, Fraser PE, Kong X, Hultin PG, Szarek WA. Arresting amyloidosis in vivo using small-molecule anionic sulphonates or sulphates: implications for Alzheimer's disease. Nat Med 1995;1:143–148. [PubMed: 7585011]
16. Dember LM, Hawkins PN, Hazenberg BP, Gorevic PD, Merlini G, Butrimiene I, Livneh A, Lesnyak O, Puechal X, Lachmann HJ, Obici L, Balshaw R, Garceau D, Hauck W, Skinner M. Eprodisate for the treatment of renal disease in AA amyloidosis. N Engl J Med 2007;356:2349–2360. [PubMed: 17554116]
17. Kisilevsky R, Gruys E, Shirahama T. Does amyloid enhancing factor (AEF) exist? Is AEF a single biological entity? Amyloid 1995;2:128–133.
18. Lundmark K, Westermark GT, Olsen A, Westermark P. Protein fibrils in nature can enhance amyloid protein A amyloidosis in mice: Cross-seeding as a disease mechanism. Proc Natl Acad Sci U S A 2005;102:6098–6102. [PubMed: 15829582]
19. Elliott-Bryant R, Cathcart ES. Amyloid enhancing factor and dietary transmission in accelerated amyloid A amyloidosis. Clin Immunol Immunopathol 1998;88:65–69. [PubMed: 9683551]

20. Solomon A, Richey T, Murphy CL, Weiss DT, Wall JS, Westermark GT, Westermark P. Amyloidogenic potential of foie gras. Proc Natl Acad Sci U S A. 2007
21. Wall JS, Richey T, Allen A, Donnell R, Kennel SJ, Solomon A. Quantitative tomography of early-onset spontaneous AA amyloidosis in interleukin 6 transgenic mice. Comp Med 2008;58:542–550. [PubMed: 19149411]
22. Li JP, Galvis ML, Gong F, Zhang X, Zcharia E, Metzger S, Vlodavsky I, Kisilevsky R, Lindahl U. In vivo fragmentation of heparan sulfate by heparanase overexpression renders mice resistant to amyloid protein A amyloidosis. Proc Natl Acad Sci U S A 2005;102:6473–6477. [PubMed: 15843464]
23. Maeda S. Use of genetically altered mice to study the role of serum amyloid P component in amyloid deposition. Amyloid 2003;10(Suppl 1):17–20. [PubMed: 14640037]
24. Botto M, Hawkins PN, Bickerstaff MC, Herbert J, Bygrave AE, McBride A, Hutchinson WL, Tennent GA, walport mj, Pepys MB. Amyloid deposition is delayed in mice with targeted deletion of the serum amyloid P component gene. Nat Med 1997;3:855–859. [PubMed: 9256275]
25. Takeda T, Hosokawa M, Higuchi K, Hosono M, Akiguchi I, Katoh H. A novel murine model of aging, Senescence-Accelerated Mouse (SAM). Arch Gerontol Geriatr 1994;19:185–192. [PubMed: 15374284]
26. Wright MD, Calkins E, Breen W, Stolte G, Schultz RT. Relationship of amyloid to aging. Review of the Literature and systematic study of 83 patients derived from a general hospital population. Medicine 1969;48:39–60. [PubMed: 4178908]
27. Higuchi K, Kogishi K, Wang J, Xia C, Chiba T, Matsushita T, Hosokawa M. Accumulation of pro-apolipoprotein A-II in mouse senile amyloid fibrils. Biochem J 1997;325(Pt 3):653–659. [PubMed: 9271085]
28. Higuchi K, Kogishi K, Wang J, Chen X, Chiba T, Matsushita T, Hoshii Y, Kawano H, Ishihara T, Yokota T, Hosokawa M. Fibrilization in mouse senile amyloidosis is fibril conformation- dependent. Lab Invest 1998;78:1535–1542. [PubMed: 9881953]
29. Higuchi K, Kitado H, Kitagawa K, Kogishi K, Naiki H, Takeda T. Development of congenic strains of mice carrying amyloidogenic apolipoprotein A-II (Apoa2c). Apoa2c reduces the plasma level and the size of high density lipoprotein. FEBS Lett 1993;317:207–210. [PubMed: 8381093]
30. Yan J, Fu X, Ge F, Zhang B, Yao J, Zhang H, Qian J, Tomozawa H, Naiki H, Sawashita J, Mori M, Higuchi K. Cross-seeding and cross-competition in mouse apolipoprotein A-II amyloid fibrils and protein A amyloid fibrils. Am J Pathol 2007;171:172–180. [PubMed: 17591964]
31. Xing Y, Nakamura A, Chiba T, Kogishi K, Takatoshi M, Zhanjun F. Transmission of Mouse Senile Amyloidosis. Lab Invest 2008;81:493–499. [PubMed: 11304568]
32. Korenaga T, Yan J, Sawashita J, Matsushita T, Naiki H, Hosokawa M, Mori M, Higuchi K, Fu X. Transmission of amyloidosis in offspring of mice with AApoAII amyloidosis. Am J Pathol 2006;168:898–906. [PubMed: 16507905]
33. Jarrett JT, Lansbury PT Jr. Seeding “one-dimensional crystallization” of amyloid: a pathogenic mechanism in Alzheimer's disease and scrapie. Cell 1993;73:1055–1058. [PubMed: 8513491]
34. Harper JD, Lansbury PT Jr. Models of amyloid seeding in Alzheimer's disease and scrapie: mechanistic truths and physiological consequences of the time-dependent solubility of amyloid proteins. Annu Rev Biochem 1997;66:385–407. [PubMed: 9242912]
35. Martin-Campos JM, Escola-Gil JC, Ribas V, Blanco-Vaca F. Apolipoprotein A-II, genetic variation on chromosome 1q21-q24, and disease susceptibility. Curr Opin Lipidol 2004;15:247–253. [PubMed: 15166779]
36. Hill AF, Desbruslais M, Joiner S, Sidle KC, Gowland I, Collinge J, Doey LJ, Lantos P. The same prion strain causes vCJD and BSE. Nature 1997;389:448–50. [PubMed: 9333232]526
37. Prusiner SB. Prions. Proc Natl Acad Sci U S A 1998;95:13363–13383. [PubMed: 9811807]
38. Chiesa R, Piccardo P, Quaglio E, Drisaldi B, Si-Hoe SL, Takao M, Ghetti B, Harris DA. Molecular distinction between pathogenic and infectious properties of the prion protein. J Virol 2003;77:7611–7622. [PubMed: 12805461]
39. Prusiner SB, Groth D, Serban A, Koehler R, Foster D, Torchia M, Burton D, Yang SL, DeArmond SJ. Ablation of the prion protein (PrP) gene in mice prevents scrapie and facilitates production of anti-PrP antibodies. Proc Natl Acad Sci U S A 1993;90:10608–10612. [PubMed: 7902565]

40. Hrncic R, Wall J, Wolfenbarger DA, Murphy CL, Schell M, Weiss DT, Solomon A. Antibody-mediated resolution of light chain-associated amyloid deposits. Am J Pathol 2000;157:1239–1246. [PubMed: 11021828]
41. Solomon A, Weiss DT, Wall JS. Immunotherapy in systemic primary (AL) amyloidosis using amyloid-reactive monoclonal antibodies. Cancer Biother Radiopharm 2003;18:853–860. [PubMed: 14969598]
42. Takaoka Y, Ohta M, Miyakawa K, Nakamura O, Suzuki M, Takahashi K, Yamamura K, Sakaki Y. Cysteine 10 Is a Key Residue in Amyloidogenesis of Human Transthyretin Val30Met. Am J Pathol 2004;164:337–345. [PubMed: 14695346]
43. Fagan AM, Watson M, Parsadanian M, Bales KR, Paul SM, Holtzman DM. Human and murine ApoE markedly alters A beta metabolism before and after plaque formation in a mouse model of Alzheimer's disease. Neurobiol Dis 2002;9:305–318. [PubMed: 11950276]
44. Ueda M, Ando Y, Hakamata Y, Nakamura M, Yamashita T, Obayashi K, Himeno S, Inoue S, Sato Y, Kaneko T, Takamune N, Misumi S, Shoji S, Uchino M, Kobayashi E. A transgenic rat with the human ATTR V30M: A novel tool for analyses of ATTR metabolisms. Biochem Biophys Res Commun. 2006
45. Morley JF, Morimoto RI. Regulation of longevity in Caenorhabditis elegans by heat shock factor and molecular chaperones. Mol Biol Cell 2004;15:657–664. [PubMed: 14668486]
46. Fortini ME, Bonini NM. Modeling human neurodegenerative diseases in Drosophila: on a wing and a prayer. Trends Genet 2000;16:161–167. [PubMed: 10729831]
47. Gidalevitz T, Ben Zvi A, Ho KH, Brignull HR, Morimoto RI. Progressive disruption of cellular protein folding in models of polyglutamine diseases. Science 2006;311:1471–1474. [PubMed: 16469881]
48. Reixach N, et al. Human-murine transthyretin heterotetramers are kinetically stable and non-amyloidogenic: A lesson in the generation of transgenic models of diseases involving oligomeric proteins. J Biol Chem 2008;283:2098–2107. [PubMed: 18006495]
49. Foss TR, Wiseman RL, Kelly JW. The pathway by which the tetrameric protein transthyretin dissociates. Biochemistry 2005;44:15525–15533. [PubMed: 16300401]
50. Takaoka Y, Tashiro F, Yi S, Maeda S, Shimada K, Takahashi K, Sakaki Y, Yamamura K. Comparison of amyloid deposition in two lines of transgenic mouse that model familial amyloidotic polyneuropathy, type I. Transgenic Res 1997;6:261–269. [PubMed: 9232027]
51. Nagata Y, Tashiro F, Yi S, Murakami T, Maeda S, Takahashi K, Shimada K, Okamura H, Yamamura K. A 6-kb upstream region of the human transthyretin gene can direct developmental, tissue-specific, and quantitatively normal expression in transgenic mouse. J Biochem 1995;117:169–175. [PubMed: 7775385]
52. Inoue S, Ohta M, Li Z, Zhao G, Takaoka Y, Sakashita N, Miyakawa K, Takada K, Tei H, Suzuki M, Masuoka M, Sakaki Y, Takahashi K, Yamamura K. Specific pathogen free conditions prevent transthyretin amyloidosis in mouse models. Transgenic Res 2008;17:817–826. [PubMed: 18357510]
53. Noguchi H, Ohta M, Wakasugi S, Noguchi K, Nakamura N, Nakamura O, Miyakawa K, Takeya M, Suzuki M, Nakagata N, Urano T, Ono T, Yamamura K. Effect of the intestinal flora on amyloid deposition in a transgenic mouse model of familial amyloidotic polyneuropathy. Exp Anim 2002;51:309–316. [PubMed: 12221923]
54. Qian X, Samadani U, Porcella A, Costa RH. Decreased expression of hepatocyte nuclear factor 3 alpha during the acute-phase response influences transthyretin gene transcription. Mol Cell Biol 1995;15:1364–1376. [PubMed: 7862129]
55. Teng MH, Yin JY, Vidal R, Ghiso J, Kumar A, Rabenou R, Shah A, Jacobson DR, Tagoe C, Gallo G, Buxbaum J. Amyloid and nonfibrillar deposits in mice transgenic for wild-type human transthyretin: a possible model for senile systemic amyloidosis. Lab Invest 2001;81:385–396. [PubMed: 11310831]
56. Sousa MM, Cardoso I, Fernandes R, Guimaraes A, Saraiva MJ. Deposition of transthyretin in early stages of familial amyloidotic polyneuropathy: evidence for toxicity of nonfibrillar aggregates. Am J Pathol 2001;159:1993–2000. [PubMed: 11733349]
57. Tagoe CE, French D, Gallo G, Buxbaum JN. Amyloidogenesis is neither accelerated nor enhanced by injections of preformed fibrils in mice transgenic for wild-type human transthyretin: the question of infectivity. Amyloid 2004;11:21–26. [PubMed: 15185495]

58. Wei L, Kawano H, Fu X, Cui D, Ito S, Yamamura K, Ishihara T, Tokuda T, Higuchi K, Maeda S. Deposition of transthyretin amyloid is not accelerated by the same amyloid in vivo. Amyloid 2004;11:113–120. [PubMed: 15478467]
59. Tagoe CE, Reixach N, Friske L, Mustra D, French D, Gallo G, Buxbaum JN. In vivo stabilization of mutant human transthyretin in transgenic mice. Amyloid 2007;14:227–236. [PubMed: 17701470]
60. Hammarstrom P, Schneider F, Kelly JW. Trans-suppression of misfolding in an amyloid disease. Science 2001;293:2459–2462. [PubMed: 11577236]
61. Santos S, Fernandes R, Saraiva M. The heat shock response modulates transthyretin deposition in the peripheral and autonomic nervous systems. Neurobiol Aging. 2008Ahead of print
62. Waits RP, Uemichi T, Zeldenrust SR, Hull MT, Field L, Benson MD. Development of lines of transgenic mice expressing the human transthyretin Ser 84 variant. Proc III Int Symp FAP and Other TTR Disorders 1995;6:S31.
63. Benson MD. Targeted suppression of an amyloidogenic transthyretin with antisense oligonucleotides. Muscle Nerve 2006;33:609–618. [PubMed: 16421881]
64. Zhao G, Li Z, Araki K, Haruna K, Yamaguchi K, Araki M, Takeya M, Ando Y, Yamamura KI. Inconsistency between hepatic expression and serum concentration of transthyretin in mice humanized at the transthyretin locus. Genes Cells. 2008
65. Westermark P, Wernstedt C, Wilander E, Sletten K. A novel peptide in the calcitonin gene related peptide family as an amyloid fibril protein in the endocrine pancreas. Biochem Biophys Res Commun 1986;140:827–831. [PubMed: 3535798]
66. Cooper GJ, Willis AC, Clark A, Turner RC, Sim RB, Reid KB. Purification and characterization of a peptide from amyloid-rich pancreases of type 2 diabetic patients. Proc Natl Acad Sci U S A 1987;84:8628–8632. [PubMed: 3317417]
67. Westermark P, Engstrom U, Johnson KH, Westermark GT, Betsholtz C. Islet amyloid polypeptide:pinpointing amino acid residues linked to amyloid fibril formation. Proc Natl Acad Sci U S A 1990;87:5036–5040. [PubMed: 2195544]
68. Yagui K, Yamaguchi T, Kanatsuka A, Shimada F, Huang CI, Tokuyama Y, Ohsawa H, Yamamura K, Miyazaki J, Mikata A. Formation of islet amyloid fibrils in beta-secretory granules of transgenic mice expressing human islet amyloid polypeptide/amylin. Eur J Endocrinol 1995;132:487–496. [PubMed: 7711888]
69. Verchere CB, D'Alessio DA, Palmiter RD, Weir GC, Bonner-Weir S, Baskin DG, Kahn SE. Islet amyloid formation associated with hyperglycemia in transgenic mice with pancreatic beta cell expression of human islet amyloid polypeptide. Proc Natl Acad Sci U S A 1996;93:3492–3496. [PubMed: 8622964]
70. Janson J, Soeller WC, Roche PC, Nelson RT, Torchia AJ, Kreutter DK, Butler PC. Spontaneous diabetes mellitus in transgenic mice expressing human islet amyloid polypeptide. Proc Natl Acad Sci U S A 1996;93:7283–7288. [PubMed: 8692984]
71. Fox N, Schrementi J, Nishi M, Ohagi S, Chan SJ, Heisserman JA, Westermark GT, Leckstrom A, Westermark P, Steiner DF. Human islet amyloid polypeptide transgenic mice as a model of non-insulin-dependent diabetes mellitus (NIDDM). FEBS Lett 1993;323:40–44. [PubMed: 8495745]
72. Vidal J, Bruce VC, Andrikopoulos S, Wang F, Hull RL, Cnop M, Olin KL, LeBoeuf RC, O'Brien KD, Chait A, Kahn SE. The effect of apolipoprotein E deficiency on islet amyloid deposition in human islet amyloid polypeptide transgenic mice. Diabetologia 2003;46:71–79. [PubMed: 12637985]
73. Soeller WC, Janson J, Hart SE, Parker JC, Carty MD, Stevenson RW, Kreutter DK, Butler PC. Islet amyloid-associated diabetes in obese A(vy)/a mice expressing human islet amyloid polypeptide. Diabetes 1998;47:743–750. [PubMed: 9588445]
74. Couce M, Kane LA, O'Brien TD, Charlesworth J, Soeller W, McNeish J, Kreutter D, Roche P, Butler PC. Treatment with growth hormone and dexamethasone in mice transgenic for human islet amyloid polypeptide causes islet amyloidosis and beta-cell dysfunction. Diabetes 1996;45:1094–1101. [PubMed: 8690157]
75. Verchere CB, D'Alessio DA, Wang S, Andrikopoulos S, Kahn SE. Transgenic overproduction of islet amyloid polypeptide (amylin) is not sufficient for islet amyloid formation. Horm Metab Res 1997;29:311–316. [PubMed: 9230354]

76. Janson J, Ashley RH, Harrison D, McIntyre S, Butler PC. The mechanism of islet amyloid polypeptide toxicity is membrane disruption by intermediate-sized toxic amyloid particles. Diabetes 1999;48:491–498. [PubMed: 10078548]
77. Kiuru S. Familial amyloidosis of the Finnish type (FAF). A clinical study of 30 patients. Acta Neurol Scand 1992;86:346–353. [PubMed: 1333716]
78. Page LJ, Suk JY, Huff ME, Lim HJ, Venable J, Yates J, Kelly JW, Balch WE. Metalloendoprotease cleavage triggers gelsolin amyloidogenesis. EMBO J 2005;24:4124–4132. [PubMed: 16281052]
79. Sugarman MC. Inclusion body myositis-like phenotype induced by transgenic overexpression of beta APP in skeletal muscle. Proc Natl Acad Sci U S A 2002;99:6334–6339. [PubMed: 11972038]
80. Perlmutter DH. Alpha-1-antitrypsin deficiency. Semin Liver Dis 1998;18:217–225. [PubMed: 9773422]
81. Geller SA, Nichols WS, Dycaico MJ, Felts KA, Sorge JA. Histopathology of alpha 1-antitrypsin liver disease in a transgenic mouse model. Hepatol 1990;12:40–47.
82. Teckman JH, Perlmutter DH. Retention of mutant alpha(1)-antitrypsin Z in endoplasmic reticulum is associated with an autophagic response. Am J Physiol Gastrointest Liver Physiol 2000;279:G961–G974. [PubMed: 11052993]
83. Burrows JA, Willis LK, Perlmutter DH. Chemical chaperones mediate increased secretion of mutant alpha 1-antitrypsin (alpha 1-AT) Z: A potential pharmacological strategy for prevention of liver injury and emphysema in alpha 1-AT deficiency. Proc Natl Acad Sci U S A 2000;97:1796–1801. [PubMed: 10677536]
84. Howlett DR, Richardson JC. The pathology of APP transgenic mice: a model of Alzheimer's disease or simply overexpression of APP? Histol Histopathol 2009;24:83–100. [PubMed: 19012248]
85. Emborg ME. Evaluation of animal models of Parkinson's disease for neuroprotective strategies. J Neurosci Methods 2004;139:121–143. [PubMed: 15488225]
86. Meredith GE, Sonsalla PK, Chesselet MF. Animal models of Parkinson's disease progression. Acta Neuropathol. 2008
87. Heng MY, Detloff PJ, Albin RL. Rodent genetic models of Huntington disease. Neurobiol Dis 2008;32:1–9. [PubMed: 18638556]
88. Liscic RM, Grinberg LT, Zidar J, Gitcho MA, Cairns NJ. ALS and FTLD: two faces of TDP-43 proteinopathy. Eur J Neurol 2008;15:772–780. [PubMed: 18684309]
89. Brandt R, Hundelt M, Shahani N. Tau alteration and neuronal degeneration in tauopathies: mechanisms and models. Biochim Biophys Acta 2005;1739:331–354. [PubMed: 15615650]
90. Duyckaerts C, Potier MC, Delatour B. Alzhemeimer disease models and human neuropathology: similarities and differences. Acta Neuropathol 2008;115:5–38. [PubMed: 18038275]
91. Newman M. Alzheimer disease: amyloidogenesis, the presenilins and animal models. Biochim Biophys Acta 2007;1772:285–297. [PubMed: 17208417]
92. Ryman D, Lamb BT. Genetic and Evnivronmental Modifiers of Alzheimer's Disease Pheotypes in the Mouse. Curr Alzheimer Res 2007;3:465–473. [PubMed: 17168645]
93. Torres-Aleman I. Mouse models of Alzheimer's dementia: current concepts and new trends. Endocrinol 2008;149:5952–5957.
94. Gimenez-Llort L, Blazquez G, Canete T, Johansson B, Oddo S, Tobena A, LaFerla F, Fernandez-Teruel A. Modeling behavioral and neuronal symptoms of Alzheimer's disease in mice: A role for intraneuronal amyloid. Neurosci Biobehav Rev 2007;31:125–147. [PubMed: 17055579]
95. Bolmont T. Induction of Tau Pathology by Intracerebral Infusion of Amyloid- -Containing Brain Extract and by Amyloid- Deposition in APP x Tau Transgenic Mice. Am J Pathol 2007;171:2012–2020. [PubMed: 18055549]
96. Oddo S, Caccamo A, Kitazawa M, Tseng BP, LaFerla FM. Amyloid deposition precedes tangle formation in a triple transgenic model of Alzheimer's disease. Neurobiol Aging 2003;24:1063–1070. [PubMed: 14643377]
97. Carlson GA, Borchelt DR, Dake A, Turner S, Danielson V, Coffin JD, Eckman C, Meiners J, Nilsen SP, Younkin SG, Hsiao KK. Genetic modification of the phenotypes produced by amyloid precursor protein overexpression in transgenic mice. Hum Mol Genet 1997;6:1951–1959. [PubMed: 9302276]

98. Sousa A, Andersson R, Drugge U, Holmgren G, Sandgren O. Familial amyloidotic polyneuropathy in Sweden: geographical distribution, age of onset, and prevalence. Hum Hered 1993;43:288–294. [PubMed: 8406517]

99. Soares ML, Coelho T, Sousa A, Batalov S, Conceicao I, Sales-Luis ML, Ritchie MD, Williams SM, Nievergelt CM, Schork NJ, Saraiva MJ, Buxbaum JN. Susceptibility and modifier genes in Portuguese transthyretin V30M amyloid polyneuropathy: complexity in a single-gene disease. Hum Mol Genet 2005;14:543–553. [PubMed: 15649951]

100. Soares ML, Coelho T, Sousa A, Holmgren G, Saraiva MJ, Kastner DL, Buxbaum JN. Haplotypes and DNA sequence variation within and surrounding the transthyretin gene: genotype-phenotype correlations in familial amyloid polyneuropathy (V30M) in Portugal and Sweden. Eur J Hum Genet 2004;12:225–237. [PubMed: 14673473]

101. Lim GP, Calon F, Morihara T, Yang F, Teter B, Ubeda O, Salem N Jr. Frautschy SA, Cole GM. A diet enriched with the omega-3 fatty acid docosahexaenoic acid reduces amyloid burden in an aged Alzheimer mouse model. J Neurosci 2005;25:3032–3040. [PubMed: 15788759]

102. Herring A, Yasin H, Ambree O, Sachser N, Paulus W, Keyvani K. Environmental enrichment counteracts Alzheimer's neurovascular dysfunction in TgCRND8 mice. Brain Pathol 2008;18:32–39. [PubMed: 17924982]

103. Wahlbom M, Wang X, Lindstrom V, Carlemalm E, Jaskolski M, Grubb A. Fibrillogenic oligomers of human cystatin C are formed by propagated domain swapping. J Biol Chem 2007;282:18318–18326. [PubMed: 17470433]

104. Kaeser SA, Herzig MC, Coomaraswamy J, Kilger E, Selenica ML, Winkler DT, Staufenbiel M, Levy E, Grubb A, Jucker M. Cystatin C modulates cerebral beta-amyloidosis. Nat Genet 2007;39:1437–1439. [PubMed: 18026102]

105. Vidal R, Frangione B, Rostagno A, Mead S, Revesz T, Plant G, Ghiso J. A stop-codon mutation in the BRI gene associated with familial British dementia. Nature 1999;399:776–781. [PubMed: 10391242]

106. Holton JL, Lashley T, Ghiso J, Braendgaard H, Vidal R, Guerin CJ, Gibb G, Hanger DP, Rostagno A, Anderton BH, Strand C, Ayling H, Plant G, Frangione B, Bojsen-Moller M, Revesz T. Familial Danish dementia: a novel form of cerebral amyloidosis associated with deposition of both amyloid-Dan and amyloid-beta. J Neuropathol Exp Neurol 2002;61:254–267. [PubMed: 11895040]

107. Pickford F, Coomaraswamy J, Jucker M, McGowan E. Modeling familial British dementia in transgenic mice. Brain Pathol 2006;16:80–85. [PubMed: 16612985]

108. Vidal R, Barbeito AG, Miravalle L, Ghetti B. Cerebral amyloid angiopathy and parenchymal amyloid deposition in transgenic mice expressing the Danish mutant form of human BRI2. Brain Pathol 2009;19:58–68. [PubMed: 18410407]

109. Kim J, Miller VM, Levites Y, Jansen-West K, Zwizinski CW, Moore BD, Troendle FJ, Bann M, Verbeeck C, Price RW, Smithson L, Sonada L, Wagg K, Rangachari V, Zou F, Younkin SG, Graff-Radford N, Dickson D, Rosenberry T, Golde TE. BRI2 (*ITM2b*) Inhibits Abeta Deposition in Vivo. J Neurosci 2008;28:6030–6036. [PubMed: 18524908]

110. Matsuda S, Giliberto L, Matsuda Y, McGowan EM, D'Adamio L. BRI2 inhibits amyloid beta-peptide precursor protein processing by interfering with the docking of secretases to the substrate. J Neurosci 2008;28:8668–8676. [PubMed: 18753367]

111. Matsuda S, Giliberto L, Matsuda Y, Davies P, McGowan E, Pickford F, Ghiso J, Frangione B, D'Adamio L. The familial dementia BRI2 gene binds the Alzheimer gene amyloid-beta precursor protein and inhibits amyloid-beta production. J Biol Chem 2005;280:28912–28916. [PubMed: 15983050]

112. Davis RL, Shrimpton AE, Holohan PD, Bradshaw C, Feiglin D, Collins GH, Sonderegger P, Kinter J, Becker LM, Lacbawan F, Krasnewich D, Muenke M, Lawrence DA, Yerby MS, Shaw CM, Gooptu B, Elliott PR, Finch JT, Carrell RW, Lomas DA. Familial dementia caused by polymerization of mutant neuroserpin. Nature 1999;401:376–379. [PubMed: 10517635]

113. Kinghorn KJ, Crowther DC, Sharp LK, Nerelius C, Davis RL, Chang HT, Green C, Gubb DC, Johansson J, Lomas DA. Neuroserpin binds Abeta and is a neuroprotective component of amyloid plaques in Alzheimer disease. J Biol Chem 2006;281:29268–29277. [PubMed: 16849336]

114. Hirko AC, Meyer EM, King MA, Hughes JA. Peripheral transgene expression of plasma gelsolin reduces amyloid in transgenic mouse models of Alzheimer's disease. Mol Ther 2007;15:1623–1629. [PubMed: 17609655]

115. Chauhan V, Ji L, Chauhan A. Anti-amyloidogenic, anti-oxidant and anti-apoptotic role of gelsolin in Alzheimer's disease. Biogerontology 2008;9:381–389. [PubMed: 18704746]

116. Liu L, Murphy RM. Kinetics of Inhibition of beta-Amyloid Aggregation by Transthyretin. Biochemistry 2006;45:15702–15709. [PubMed: 17176092]

117. Schwarzman, AL.; Goldgaber, D. Interaction of transthyretin with amyloid Beta-protein: binding and inhibition of amyloid formation. In: Bock, Gregory R.; Goode, Jamie A., editors. The Nature and Origin of Amyloid Fibrils. John Wiley & Sons; New York: 1996. p. 146-164.

118. Buxbaum JN, Ye Z, Reixach N, Friske L, Levy C, Das P, Golde T, Masliah E, Roberts AR, Bartfai T. Transthyretin protects Alzheimer's mice from the behavioral and biochemical effects of Abeta toxicity. Proc Natl Acad Sci U S A 2008;105:2681–2686. [PubMed: 18272491]

119. Choi SH, Leight SN, Lee VM, Li T, Wong PC, Johnson JA, Saraiva MJ. Accelerated Abeta deposition in APPswe/PS1deltaE9 mice with hemizygous deletions of TTR (transthyretin). J Neurosci 2007;27:7006–7010. [PubMed: 17596449]

Table 1

Amyloid fibril proteins and their precursors in human[#]

Amyloid protein	Precursor	Systemic (S) or Localized, organ restricted (L)	Syndrome or Involved Tissues
AL	Immunoglobulin light chain	S, L	Primary
			Myeloma-associated
AH	Immunoglobulin heavy chain	S, L	Primary
			Myeloma-associated
$A\beta_2M$	β_2-microglobulin	S	Hemodialysis-associated
		L?	Joints
ATTR	Transthyretin	S	Familial
			Senile systemic
		L?	Tenosynovium
AA	(Apo)serum AA	S	Secondary, reactive
AApoAI	Apolipoprotein AI	S	Familial
		L	Aorta, meniscus
AApoAII	Apolipoprotein AII	S	Familial
AApoAIV	Apolipoprotein AIV	S	Sporadic, associated with aging
AGel	Gelsolin	S	Familial (Finnish)
ALys	Lysozyme	S	Familial
AFib	Fibrinogen α-chain	S	Familial
ACys	Cystatin C	S	Familial
ABri	ABriPP	S	Familial dementia, British
ADan*	ADanPP	L	Familial dementia, Danish
Aβ	Aβ protein precursor (AβPP)	L	Alzheimer's disease, aging
APrP	Prion protein	L	Spongioform encephalopathies
ACal	(Pro)calcitonin	L	C-cell thyroid tumors
AIAPP	Islet amyloid polypeptide**	L	Islets of Langerhans
			Insulinomas
AANF	Atrial natriuretic factor	L	Cardiac atria
APro	Prolactin	L	Aging pituitary
			Prolactinomas
AIns	Insulin	L	Iatrogenic
AMed	Lactadherin	L	Senile aortic, media
AKer	Kerato-epithelin	L	Cornea, familial
ALac	Lactoferrin	L	Cornea
AOAAP	Odontogenic ameloblast-associated protein	L	Odontogenic tumors
ASemI	Semenogelin I	L	Vesicula seminalis
ATau	Tau	L	Alzheimer's disease, fronto-temporal dementia, aging, other cerebral conditions

[#]Proteins are listed, when possible, according to relationship. Thus, apolipoproteins are grouped together, as are polypeptide hormones

* ADan comes from the same gene as ABri

** Also called ‘amylin’