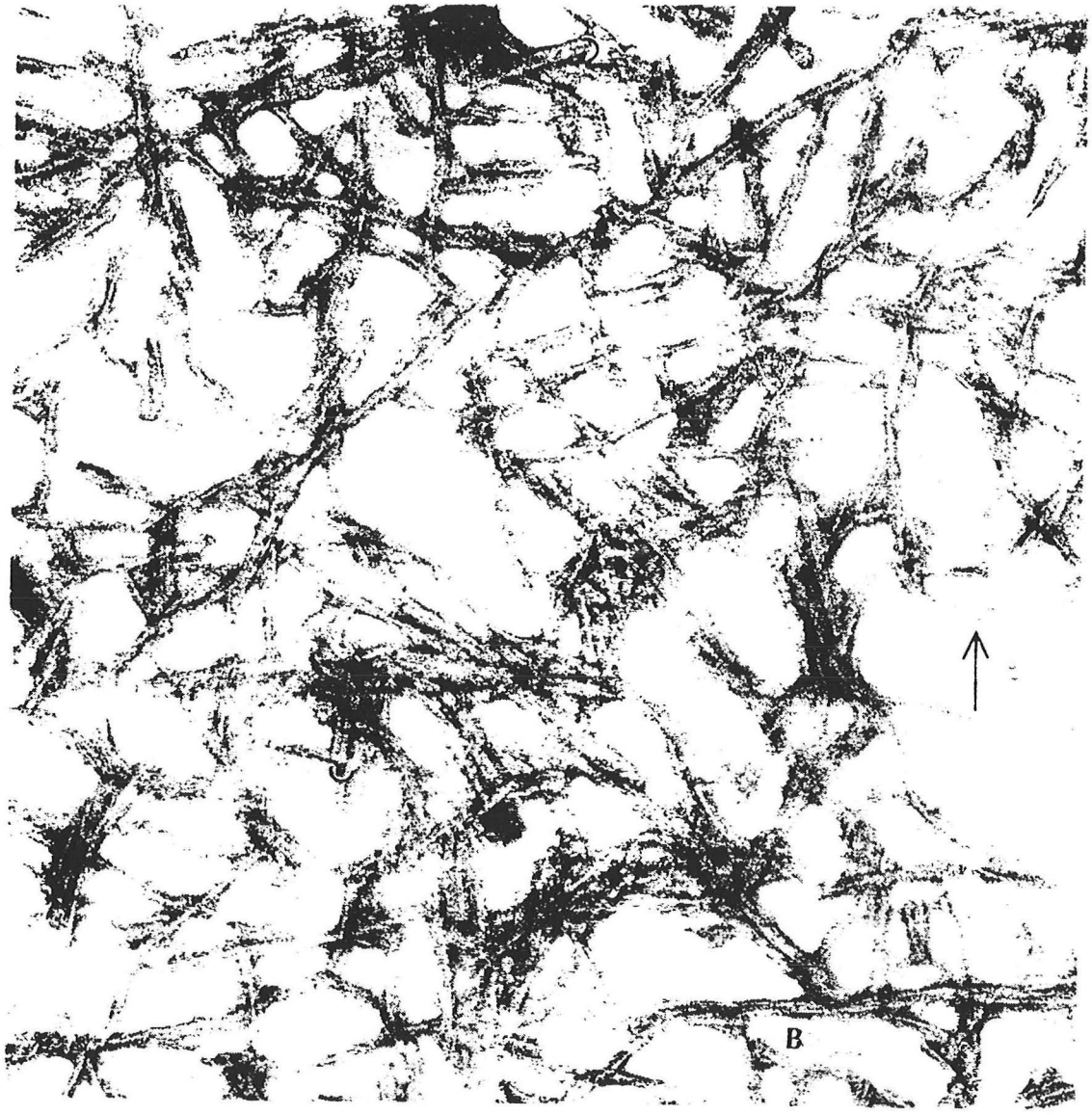
AMYLOIDOSIS

Diseases of Misfolded Proteins



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INTRODUCTION

Amyloidosis is a broad term for a heterogeneous group of diseases that have in common the extracellular deposition of insoluble fibrillar proteins. The deposited proteins are the cause of the disease and are unique for each type of amyloid. All share a β-pleated-sheet configuration and green birefringence on polarization microscopy after Congo red staining (1).

History

Amyloidosis has occurred for centuries, but it was not until the mid-1800s that Virchow showed the lardaceous tissues which others had described, stained with iodine and sulfuric acid. He coined the term "amyloid" which means starch-like because of this reaction which led him to believe that the amyloid represented deposits of carbohydrates. Several years later, Friedreich and Kekule showed that the amyloid deposits were composed mainly of protein (1). Subsequently, it was found that amyloidosis was often associated with chronic diseases, particularly those that were suppurative. It was also appreciated that some patients with amyloidosis had no apparent predisposing illness and these individuals were diagnosed with "primary amyloidosis". Later, several families were described with amyloidosis. Eventually, the term "primary" was applied to the sporadic forms of amyloidosis and "secondary" to amyloidosis that developed in patients with chronic inflammatory diseases such as tuberculosis, rheumatoid arthritis, and osteomyelitis. Hereditary amyloidosis was not well accepted until Andrady published the findings of Portuguese families with a polyneuropathy (2).

Characterization of Amyloid

Characterization of amyloid remained relatively descriptive until 1959 when Cohen, Calkins and Spiro showed by electron microscopy that amyloid deposits were not amorphous collections of proteinatous material, but rather contained non-branching fibrils with diameters of seventy-five to one-hundred Å and were of indeterminant length (3,4). The origin and nature of amyloid fibers remained a mystery because of difficulty in solubilizing the highly resistant fibers. However, in 1971, Glenner, using strong chaotropic agents, solubilized amyloid material from patients with the primary form of amyloidosis (5). Amino acid sequencing revealed that these subunits were homolous to the variable segment of immunoglobulin light chains. This breakthrough was soon followed by the solubilization of amyloid from patients with the so-called "secondary" form of amyloidosis. The composition of these fibers resembled those of an acute phase reactive plasma protein which was elevated by inflammation. This protein was then given the name "serum amyloid A protein" or "SAA" (6,7). In 1978, it was discovered that the hereditary forms of amyloid resembled a protein termed prealbumin which was later found to be plasma transthyretin, a protein that transports in the serum both thyroxine and retinol binding protein (8). Later, in other families with hereditary forms of amyloidosis, other proteins were implicated including apolipoprotein A1, gelsolin, and the α-chain of fibrinogen.

Amyloidosis as a disease frequently escapes detection and recognition by clinicians and is often diagnosed unsuspectingly by biopsy of an involved organ or at post-mortem examination. Today, I

would like to review the various forms of systemic amyloidosis, both from the basic principles, and the clinical presentations.

Properties of Amyloid Fibrils

Amyloid is defined by its reaction to staining with Congo red and its other histologic properties as shown in Table 1. Microscopically, the fibers appear to be red in normal light when stained with alkaline Congo red, but change to an apple-green when viewed in a polarized light. The deposits are extracellular and appear to be crowding the tissue cells leading to their eventual death perhaps by apotosis or programmed cell death (9). The relentless accumulation of this fibrillar material that resists phagocytosis, proteolysis, and normal host defense mechanisms leads to death when the amyloid involves vital organs.

Table 1. ***Histologic Criteria for Definition of Amyloid***

1.	Homogeneous, hyaline, eosinophilic material on H&E
2.	Crystal Violet metachromasia
3.	Stains with alkaline Congo Red
4.	Apple green positive birefringence with polarized light after Congo Red staining
5.	Fibrillar structure on electron microscopy
6.	Stains with antibody specific for P-component
7.	Stains with antibody directed toward specific precursor

Under the electron microscope, amyloid deposits contain a characteristic fibrillar structure which are often in linear arrays, but lack an ordered structure (Fig. 1). When these fibers are physically extracted from the tissues and negatively stained with uranyl acetate and studied by electron microscopy, the non-branching fibrils appear to consist of at least two and perhaps several parallel subunit filaments. These subunits are helically twisted and they measure about twenty-five to thirty-five Å in width and have a beaded appearance (1). There are no ultrastructural features that can distinguished between the various proteins which can produce amyloidosis. The substructure of amyloid fibrils has been studied by x-ray diffraction and while these fibers basically have a crystalline structure which gives them their birefringence, it has not been possible to study the crystal lattice since it has been so difficult to solubilize and recrystallize the amyloid fibers (10,11). However, x-ray powder patterns show that the fibers all have a β-pleated-sheet structure. These fibrillar structures are not usually found in this conformational state in normal mammalian tissues (12,13). The pathological processes apparently are dependent on a specific protein conformation, the twisted β-pleated sheet fibril which represents a rather unique structure. In general, the peptide chain within the fibrils is arranged in two groups of parallel or near parallel amino acid sequences. Immunoglobulin light chain domains have an extensive anti-parallel β configuration (12). The transthyretin monomer has an extensive β structure with approximately eight polypeptide segments

running in an anti-parallel fashion in two planes as shown in Figure 2 (13). Tertiary structure of SAA protein in reactive amyloidosis is not as well understood and structural models suggest the β helices are also involved in an intrinsic fibrillar formation.

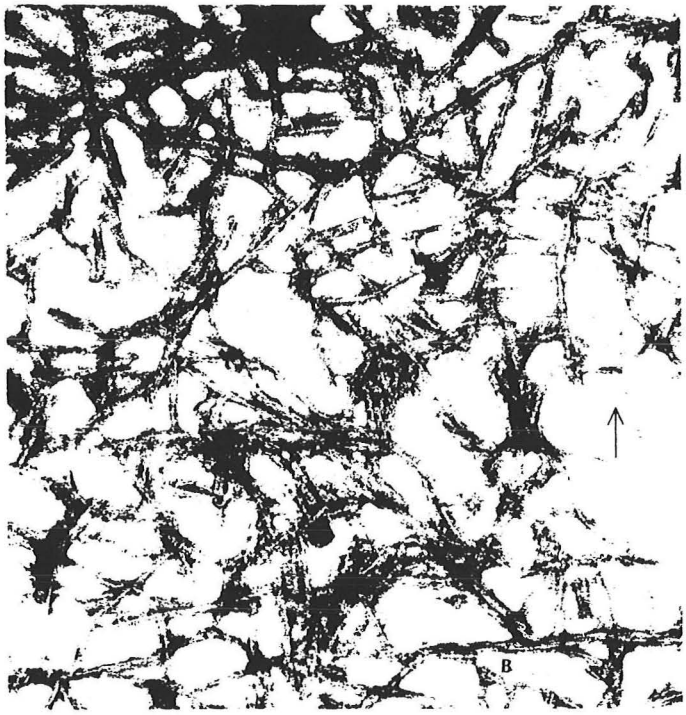

Fig. 1. EM shows isolated purified amyloid negatively stained with phosphotungstic acid. Arrow indicates single filament. Most of these end together (half circles). B is a thicker bundle of intertwined filaments (x 96,000). (Courtesy D. Zucker-Franklin; from E.C. Franklin, D. Zucker-Franklin: Adv Immunol 15:268, 1972).

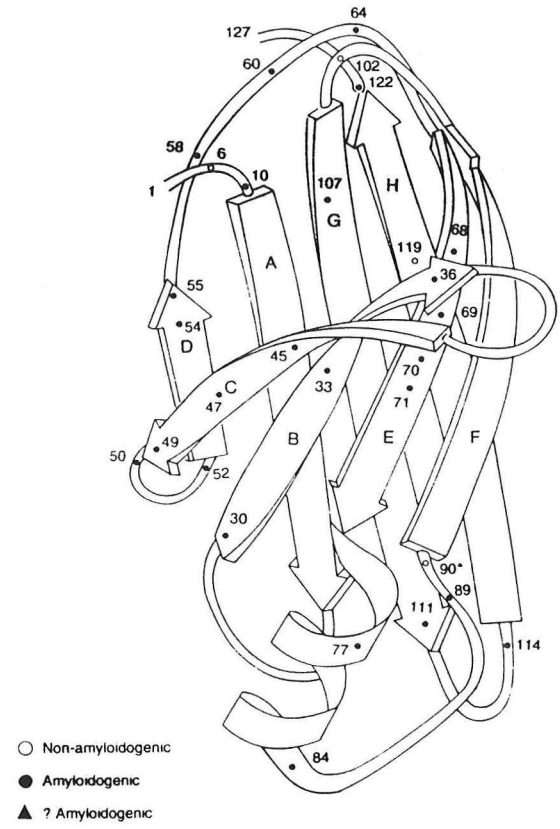

Fig. 2. A schematic drawing of the crystal structure of the transthyretin monomer with the sites of the first 29 amino acid substitutions reported. The closed circles are amyloidogenic mutations whereas the open circles are not. The total number of amyloidogenic substitutions is now more than 50.

As described previously, initially amyloidosis was classified into a primary form where there was no obvious disease process. A secondary variety in which there was inflammatory or suppurative diseases. The third being the familial or inherited form. Now it is possible with the recent advances in protein chemistry to classify amyloidosis on the basis of the involved protein as shown in Table 2. Immunoglobulin (AL) amyloidosis is either primary in that there is a plasma cell dyscrasia, but no overt multiple myeloma or an AL amyloidosis with associated myeloma. The subunit is the Ig light chains, either Kappa or Lambda and the distinguishing feature is that they are monoclonal immunoglobulins. The secondary form is better termed a reactive (AA) amyloidosis and represent

deposition of the amyloid A protein in tissues, the distinguishing feature being the presence of an inflammatory disease. The hereditary amyloidosis are comprised of a series of different proteins. In the familial forms, transthyretin is the most common mutant protein and it is inherited as an autosomal dominant disease. Hereditofamilial amyloidosis also can be caused by a mutant apolipoprotein A-1. Familial amyloidosis with a polyneuropathy may be produced by mutations in any one of several different proteins, including fibrinogen, gelsolin, and lysozyme. The fourth type of amyloidosis is the β-2 macroglobulin (β-2-M) which is observed in long-term renal dialysis patients. The protein is β-microglobulin deposition. In addition to these systemic varieties of amyloidosis, there are localized forms of the disease as shown in Table 2. These include Alzheimers, medullary carcinoma of the thyroid, Type II diabetes and isolated atrial amyloid. I intend to confine my remarks today to the systemic form of amyloidosis.

Table 2. ***Chemical Classification of the Human Amyloidoses***

Amyloid protein	Precursor protein	Clinical syndrome
AL	Immunoglobulin L-chain	Primary amyloid; multiple myeloma
AH	Immunoglobulin H-chain	Primary amyloid; multiple myeloma
AA	apo-SAA	Inflammation-associated familial Mediterranean fever; Muckle-Wells
ATTR	Transthyretin	Familial amyloidotic polyneuropathy; senile systemic amyloidosis; isolated vitreous amyloid
AGEL	Gelsolin	Familial amyloidotic polyneuropathy; Finnish lattice corneal dystrophy
AApoAI	Apolipoprotein Al	Familial amyloidotic polyneuropathy; Van Allen
$A\beta_2M$	Beta-2 microglobulin	Dialysis-associated amyloidosis
A_β	Beta-protein precursor	Alzheimers; Down syndrome; HCHWA (Dutch)
ACys	Cystatin C	HCHWA (Iceland)
ACal	Calcitonin	Medullary carcinoma of the thyroid
AIAPP	Islet amyloid polypeptide	Insulinoma; type II diabetes mellitus
AANF	Atrial natriuretic factor	Isolated atrial amyloid
AScr	Scrapie (prion) protein	Creutzfeldt-Jakob; Gerstmann-Straussler-Scheinker syndrome
AFibrin	Alpha chain fibrinogen	Hereditary renal amyloid
ALys	Lysozyme	Hereditary non-neuropathic systemic amyloidosis (Ostertag type)

HCHWA, hereditary cerebral hemorrhage with amyloidosis.

Pathogenesis

In all forms of amyloidosis, the precursor is synthesized as a soluble protein and amyloid fibrils are rarely seen inside the synthesizing cell. It has been suggested that amyloidogenic proteins are unstable and require a set of proteins called chaperonins that are often heat-shock proteins to maintain their solubility intracellularly (14, also see Ivor Benjamin's Grand Rounds, 12/18/97). Chaperones escort the proteins to the secretory pathway, or if the proteins are defective, to lysosomes for degradation into peptide fragments. The fibril precursors may be aberrant folding intermediates of monomers generated during denaturation of polymeric molecules before lysosomal proteolysis (15)(Fig. 3). Conformational alterations of amyloidogenic proteins have in common the 5-10 nm wide non-branching fibrils composed of polypeptides in the β-pleated-sheet conformation. Figure 4 shows a structural rearrangement in a mutant transthyretin molecule that is believed to convert it to

amyloidogenic conformation. Several amyloidogenic proteins including transthyretin variants, immunoglobulin light chains, lysozyme and the β peptide have been converted to amyloid fibrils by inducing in vitro conformational changes in the normally folded proteins (15,16). Amyloidosis appears to be a disease of protein folding like prions-related diseases. Normal prion proteins have little β-sheet structure. However, when the normal prion protein folds into infectious mutant prions, they fold into highly β-pleated structures like amyloid (14).

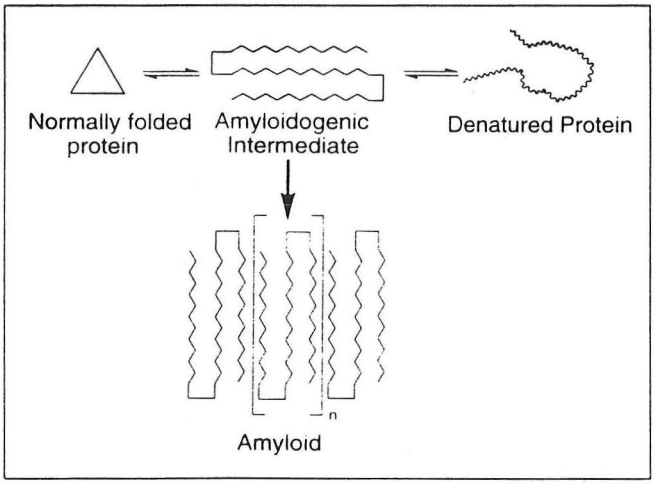

Fig. 3. A schematic representation of the competitive denaturation and fibrilogenesis pathways in several amyloidogenic proteins under partially denaturing conditions. Several single-site mutations that cause early-onset amyloid disease appear to function by shifting the equilibrium between the normally folded protein and the amyloidogenic intermediate under mildly denaturing conditions where the wild-type protein would be stable.

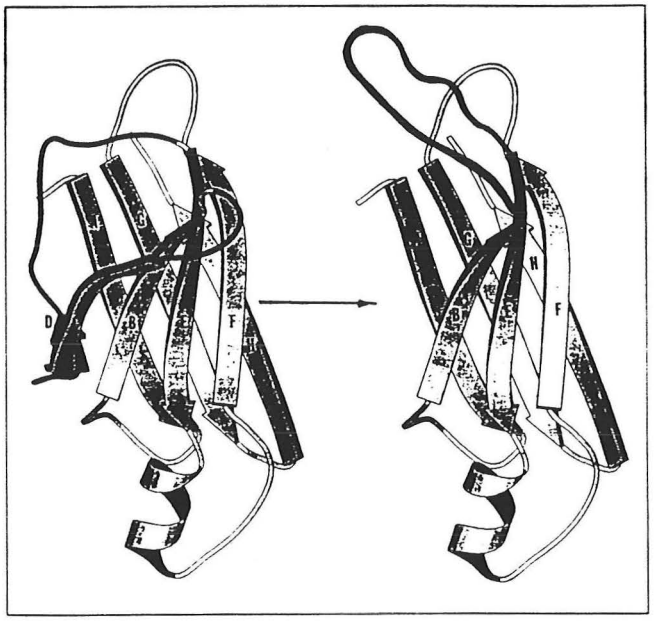

Fig. 4. A ribbon diagram representation of the structural rearrangement thought to yield the amyloidogenic intermediate of transthyretin. The C-strand-loop-D-strand region of the protein becomes a disordered loop in the amyloidogenic intermediate and is depicted by the heavy black line.

As shown in Figure 2, the amyloidogenic mutations seem to be spread throughout the transthyretin molecule and, therefore, a single physicochemical change is unlikely to explain the tendencies of these mutations to form fibrils. As shown in the figure, the A strand and H strand have the lowest densities of amyloidogenic variation (14). It is possible that the paucity of mutations in one or both of these regions reflects the fact that they may be necessary for transthyretin fibrillogenesis. Consistent with this notion is the observation that the A and H strands have core peptide properties. However, it is also true that the A and H strands are involved in dimer-dimer interactions. Hence, mutations in these regions may not be tolerated with respect to the transport functions of transthyretin. Since transthyretin is synthesized as a soluble protein by the liver, the mutations are believed to alter

denaturation before normal catabolic degradation occurs. These mutations then would render the molecules unstable in the unfolded state and, thus, subject to fibrillogenic aggregation, the so-called "off-pathway of folding". It has been suggested that the C-strand-loop-D strand conformation change allows the core peptides on adjacent molecules to interact and to form fibrils.

Another question is what determines the site of deposition of amyloid fibrils as they are not randomly deposited throughout the body. There appears to be some specificity with each of the amyloid proteins having its own pattern of deposition. However, some tissues appear to be favorite targets for several different amyloid proteins. For example, several different amyloid proteins have a predilection for binding to peripheral nerves, heart muscle, kidneys and vascular walls. Glycosaminoglycans (GAGs), heparin sulfate containing molecules, are suspected to have an important role in this deposition (17). Studies in experimentally induced murine AA amyloid indicated that small molecular weight GAG analogs reduce the rate and extent of deposition of this protein (18).

REACTIVE AMYLOIDOSIS (AA)

Etiology

Reactive (secondary) amyloidosis was the major form of the disease prior to the antibiotic era. It was associated frequently with tuberculosis, bronchiectasis, osteomyelitis, and chronic abcess formations. Today the disease is seen primarily in patients with rheumatoid arthritis, although the incidence is thought to be less than five percent. Other entities associated with reactive amyloidosis are psoriatic arthritis, granulomatous bowel disease, and Familial Mediterranean Fever. Reactive amyloidosis usually appears after several years of rheumatoid arthritis with the insidious onset of proteinuria followed by the nephrotic syndrome and hepatosplenomegaly. Reactive amyloidosis is also an important cause of morbidity and mortality in patients with the juvenile form of rheumatoid arthritis occurring in approximately four percent. The onset of reactive amyloidosis occurs at a mean of eight years after the diagnosis of juvenile arthritis. Hypernephroma and Hodgkin's disease are also tumors associated with the reactive (AA) form of amyloidosis.

Characterization of the AA Protein

The precursor protein of reactive amyloid fibrils is the serum amyloid A (SAA) which is synthesized primarily in the liver. The production of hepatic SAA are very similar to those for C-reactive protein, another acute phase reactant. Studies suggest that both of these proteins are synthesized under the direction of an inducer probably produced by macrophages. Interleukin-1 and Interleukin-6 have been shown to stimulate hepatocytes to produce SAA by inducing the transcription of the SAA genes (19). There are at least four SAA genes in humans and they are located on the short arm of chromosome 11. One of the human genes, the SAA-3 gene has become a pseudogene and is no longer expressed. Two SAA proteins, type 1 and type 2, are other acute phase reaction proteins.

The human SAA protein has 104 amino acid residues in a single polypeptide chain. The monomer is usually cleaved between residues 76 and 77 and the N-terminal 76 residues are those that are incorporated into the amyloid fibrils. It has been suggested that a defect in degradation of this cleavage product may contribute to reactive amyloidosis. Apparently the SAA 1 gene product is the predominant one in most of the reactive forms of amyloidosis.

Clinical Presentation

The organs that are usually involved in reactive amyloidosis include the kidney, the liver and the spleen. Most patients present with the nephrotic syndrome which may persist for years until azotemia occurs. Massive splenomegaly and hepatomegaly are common in the latter stages of this disease. The gastrointestinal tract may also be affected, but it is less symptomatic than the form seen with immunoglobulin (AL) amyloidosis. Cardiac and skeletal muscle are rarely involved with reactive amyloidosis. Life expectancy depends on when the diagnosis is made, but since the renal disease progresses slowly, a patient may be nephrotic for five or more years before exhibiting severe renal insufficiency.

Diagnosis

One must be cautious in biopsying organs affected by amyloidosis since bleeding has been reported, particularly following liver biopsy. The aspiration of abdominal fat is safe and highly effective in making the diagnosis. It is said to be approximately eighty-five percent sensitive with sufficient specimens and using Congo red stain (20,21). Scintigraphy with I^{131}-labeled serum amyloid P component, a nonfibrillar serum glycoprotein that binds specifically to amyloid fibers in vitro and in vivo is a technique for visualizing amyloid. It is retained in tissue amyloid deposits for days apparently protected from the normal rapid catabolism to which the P component is subject in the circulation. Therefore, the labeled amyloid P component can be used for diagnosing, locating and monitoring the extent of systemic amyloid (22).

Treatment

Therapy for AA amyloidosis is directed at the underlying cause of the acute phase reaction. Suppurative processes are treated by antibiotics and, where indicated, surgery. No treatment specifically causes the resolution of amyloid deposits, but therapy reduces the supply of AA amyloid fibril precursors and can improve survival and preserve organ function. In amyloidosis secondary to juvenile rheumatoid arthritis, treatment with chlorambucil causes regression of the AA fibrils (23). This drug suppresses the acute phase production of serum amyloid A protein, the precursor of AA fibrils and is associated with remission of proteinuria in nephrotic patients. Scintigraphy with I^{131}-labeled serum amyloid P component shows a major regression of amyloid after 12 to 36 months treatment with chlorambucil (23).

Familial Mediterranean Fever

Familial Mediterranean Fever is an inherited disease among people of the near East -- Sephardic Jews, Armenians, Turks and Arabs. It is transmitted as an autosomal recessive trait and is characterized by sporadic episodes of acute inflammation of the pleural, peritoneal and joint spaces and occasionally the pericardium and the tunica vaginalis of the testes (24). These episodes usually last only a few days, but in some patients, a chronic arthritis develops. In approximately a quarter of affected patients, renal amyloidosis develops in which the amyloid is of the serum amyloid A (AA type). The amyloidosis in these subjects will progress and nearly all deaths due to Familial Mediterranean Fever are the result of renal failure. Recently, there has been new information on the pathogenesis of this disease. Patients with Familial Mediterranean Fever are believed to lack a chemotactic-factor-inactivating enzyme that normally occurs in body fluids. It is believed that the chemotactic-factor, probably C5a, is released by injury to the serosal surfaces during normal activity, but the amounts are small enough that they are cleared promptly by the inactivating enzyme. In Familial Mediterranean Fever, the inactivating enzyme has been found to be low or absent and the chemotactic factor, that is C5a, persists and recruits neutrophils which then release a variety of products including enzymes that generate more factor C5a as well as other cytokines which induce an inflammatory response (25).

Recently, the gene responsible for Familial Mediterranean Fever has been cloned and is designated MEFV. It is a gene of approximately 10 kb with ten exons. It expresses a 3.7 kb transcript encoding 781 amino acids and the protein has been called pyrin or marenostrin. This protein is related to the RETRO-gene family and suggests that it is a transcription factor presumably regulating the expression of target genes involved in suppressing inflammation, for example, the chemotactic factor inactivator. In support of these conclusions, several mutations have been identified in exon 10 in a large number of affected patients (25). It should be noted that several Mediterranean Fever patients lack a mutation in this protein raising the question that there may be other mutations at different loci which contribute to this clinical entity. Moreover, the so-called mutations in exon 10 may be polymorphisms that are innocuous rather than disrupting the function of pyrin. More work obviously is needed.

Treatment

Colchicine has been shown to be very effective in treating patients with Familial Mediterranean Fever and can prevent the progression of renal disease in these patients. Prophylactic colchicine also reduces the frequency of the attacks as well as preventing the amyloidosis (24,26).

IMMUNOGLOBULIN AMYLOIDOSIS (AL)

Immunoglobulin amyloidosis is, at present, the most common form of systemic amyloidosis. The prevalence of this disease is not known, but a significant percentage of patients who are afflicted, die without benefit of a correct diagnosis. The protein responsible for immunoglobulin amyloidosis is the immunoglobulin light chain or their fragments which forms the amyloid fibril. This group of disorders as shown in Table 2 is heterogeneous and includes primary amyloidosis and amyloidoses associated with multiple myeloma and other plasma cell dyscrasias such as Waldenstrom's macroglobulinemia and heavy chain disease (23,27,28). The common factor in these amyloidoses is the overproduction of a monoclonal immunoglobulin protein. The light chain of this clonal product or one of its domains becomes the subunit of the amyloid fibrils. In primary amyloidosis, the initiation factors for overproduction of immunoglobulin light chains by a particular clone of B-lymphocytes or plasma cells is unknown. There may be a slight increase in the number of plasma cells seen in the bone marrow in the range of five to ten percent. These cells have a more abundant cytoplasm than normal plasma cells, but the nucleus does not exhibit the characteristics associated with a malignancy. With respect to multiple myeloma, it is the malignant nature of the cell which increases their numbers and increases the production of light chains and their fragments. Analysis of amyloid in the AL type shows that certain light chains are more amyloidogenic than others. Lambda light chains predominate in amyloid fibers with respect to Kappa chains and the ratio is approximately three to one (1,27). This is the reverse of the prevalence of Kappa and Lambda chains in normals and in patients with myelomas in which the ratio of Lambda to Kappa chains is two to three. The reasons for these amyloidogenic Lambda chains may be due to the fact that free Bence-Jones proteins in plasma exist as dimers and Lambda light chains have a higher association constant than Kappa light chains. Analysis of AL amyloid indicates that the subunit proteins contain the entire variable segment of the light chain plus approximately the first tryptic peptide of the constant region. This variable segment has extensive β-sheet structures and these are easily incorporated into the β-pleated-sheet of the amyloid fibril. It is uncertain whether digestion of the free light chains occurs in the lysosomes of phagocytic cells that release light chain fragments that then aggregate into the fibrils, or whether the light chains are incorporated into the fibrils and then the constant region is cleaved. When the composition of the AL fibers is further analyzed, it appears that the Lambda VI light chains and the Kappa I are the most common subgroups in patients with AL amyloidosis (12,27). It should be noted that the Lambda VI light chains account for less than five percent of normal immunoglobulins even though they constitute the majority of the immunoglobulin variable region light chains in the amyloid fibrils.

Support for the above conclusions was strengthened when Bence-Jones proteins were subjected to lysosomal enzyme digestion or peptic or tryptic digestion. Under these conditions, amyloid fibrils spontaneously formed in vitro. Moreover, amyloid has been reported in heavy chain disease when the protein is truncated and is essentially the same size and structure as the variable region of the light chain immunoglobulin (28,29). Since the domains of heavy and light chains are similar at the tertiary structural level, it would appear that the size of the immunoglobulin protein is a determining factor in fibril formation. The molecular weights of amyloid fibril proteins are between 15,000-16,000, again, emphasizing the importance of size in fibrillar formation.

Clinical Presentation

Immunoglobulin amyloidosis affects primarily mesenchymal derived organs such as the heart, skeletal muscle and nerve. In addition, the kidneys are commonly involved with the clinical presentation being the nephrotic syndrome. Occasional patients will present with a factor X deficiency and a coagulopathy with bleeding. It has been shown that factor X has a high affinity for amyloid fibers and noncovalently binds to them, leading to a reduction in circulating factor X (30,31). As with renal involvement in reactive amyloidosis, the progression of renal failure is relatively slow. There may be massive proteinuria with profound edema and hypoalbuminemia with retention of renal function and a prolonged delay in the development of elevated serum creatinine. Systemic hypertension is also uncommon in renal amyloidosis (1,23,27).

The heart is frequently involved with AL amyloidosis. Table 3 lists the clinical characteristics of cardiac AL. Congestive failure often appears rapidly and is progressive. The symptoms are frequently those of right heart failure with markedly elevated jugular venous pressures, hepatomegaly and peripheral edema. The electrocardiogram shows extremely low voltage with a left axis deviation and the so-called pseudo-infarct patterns in leads V1-V4 with apparent Q-waves (20). This pattern may lead the physician to diagnose arteriosclerotic coronary artery disease. Echocardiography frequently shows concentric and thickened left and right ventricle with normal to small ventricular cavities. Measurements of ejection fractions indicate that they are low-normal or mildly reduced. The general pattern of cardiac dysfunction is that of a restrictive cardiomyopathy. It should be noted that calcium channel blockers which may be given in an attempt to treat presumptive diastolic dysfunction often worsen the cardiac failure and these drugs are contraindicated. Life-threatening ventricular arrhythmias are also common with cardiac amyloid as is atrial fibrillation due to infiltration of the atria. With atrial fibrillation, thrombus formation and thromboembolism are common.

Table 3. ***Clinical Characteristics of Cardiac AL***

Dilated cardiomyopathy:	Coronary insufficiency:
Cardiomegaly	Angina pectoris
Predominant systolic dysfunction	Myocardial infarction
Restrictive cardiomyopathy:	Electrocardiographic abnormalities:
Slight cardiomegaly	Low-voltage QRS complexes in the limb leads
Predominant diastolic dysfunction	Pseudo-infarct pattern
'Stiff heart' syndrome:	Abnormalities in atrio-ventricular and intra-ventricular conduction
Thickening of the ventricular wall and septum	Rhythm disturbances:
Poorly compliant ventricles	Atrial fibrillation
Biatrial enlargement	Atrial or junctional tachycardia
Congestive heart failure	Premature ventricular complexes
Pericardial tamponade	Ventricular tachycardia
Valvular dysfunction	Sick sinus syndrome
Atrial thrombosis - embolization	Enhanced sensitivity to digitalis glycosides

The gastrointestinal tract is frequently involved in AL amyloidosis. Table 4 depicts hepatic and splenic manifestations of AL. Hepatomegaly is common as is infiltration of the gut (27). The liver is often massively enlarged, irregular and hard. The alkaline phosphatase concentration is elevated, but transaminases are usually normal or only minimally increased. Biopsy of the liver is to be avoided because of the frequency of hemorrhage. Splenomegaly is rare in AL amyloid, occurring in less than five percent of patients. Hyposplenism, however, occurs and can be identified by the presence of Howell-Jolly bodies in the peripheral blood smear. Involvement of the intestines may be accompanied by chronic diarrhea and weight loss.

Table 4. ***Clinical Manifestations of Hepatic and Splenic AL***

Liver:
Hepatomegaly (disproportional to the liver enzyme abnormalities)
Isolated elevation of alkaline phosphatase
Hyperbilirubinemia - jaundice
Intrahepatic cholestasis
Sinusoidal portal hypertension
Coagulation abnormalities
Spleen:
Splenomegaly
Functional hyposplenism:
Howell-Jolly bodies in the peripheral blood smear
Thrombocytosis
Coagulation abnormalities (Factor X deficiency)
Spontaneous rupture of the spleen

Involvement of the central nervous system is rare with AL amyloidosis, but sensory neuropathies and autonomic neuropathies are quite common. Table 5 shows the major presenting signs and symptoms of AL, many are neurologic. Carpal tunnel syndrome is often the first symptom followed by years later, a sensory neuropathy with distal to proximal progression and symmetrical patterns. The autonomic nervous system dysfunction may be severe, resulting in symptomatic postural hypotension, impotence and disturbance of gastrointestinal motility. The autonomic neuropathy complicates treatment of congestive heart failure and the nephrotic syndrome since vasodilatory drugs and diuretics may aggravate the postural hypotension (20).

Table 5. ***Major Presenting Manifestations of Systemic AL***

Weakness or fatigue	Dyspnea
Loss of weight	Skin manifestations:
Autonomic disturbances:	Purpura
Syncope	Papules, tumors
Orthostatis hypotension	Skin fragility
Peripheral neuropathy (paresthesias)	Bleeding
Horseness	Carpal tunnel syndrome
Change of voice	Hepato- and/or splenomegaly
Macroglossia	Lymphadenopathy
Edema, due to:	Occasional detection of an MIg in the serum and/or urine
Congestive heart failure	
Nephrotic syndrome	
Protein-losing enteropathy	

Infiltration of the vasculature with AL amyloid results in easy bruising. Table 6 summarizes cutaneous manifestations of systemic AL. Spontaneous periorbital hemorrhage and purpura are sometimes seen with minimal trauma such as sneezing. This is frequently termed the "raccoon-eye" sign of spontaneous periorbital purpura. An enlarged tongue or macroglossia is a well-known feature of amyloidosis that occurs in approximately twenty percent of patients. It is characterized by an enlarged and stiffened tongue that is frequently rimmed by the indentations of the teeth. AL amyloid may infiltrate the skin and give rise to the so-called "shoulder pad" sign, nail dystrophy, or rarely, alocopecia. Infiltration of the vocal chords may produce hoarseness or a weak voice (20).

Table 6. ***Cutaneous Manifestations of Systemic AL***

Infiltration of blood vessel wall:
Hemorrhagic manifestations:
Purpura
Petechiae
Ecchymoses
Cord-like thickening of cutaneous vessels
Infiltrative lesions:
Papules, nodules, plaques
Tumefactive lesions
Bullous lesions
Scleroderma-like infiltration of the face and extremities
Cutis laxa
Involvement of cutaneous annexes:
Onycopathy
Alopecia
Breast nodules

Involvement of other organs is also seen. AL amyloidosis may involve the lung, but it is rarely symptomatic. Table 7 describes the rare complications that may occur with respiratory AL. In patients with heart failure, a rapid reaccumulation of pleural effusions should alert the clinician to the possibility of pleural amyloid. Adrenal involvement may lead to hypoadrenalism which is masked because of the associated hypotension and hyponatremia attributed to the autonomic dysfunction and heart failure. It is recommended that adrenal function be assessed in patients with these findings. Infiltration of the thyroid gland may occur in 10 to 20 percent of patients and be associated with hypothyroidism.

Table 7. ***Clinical Manifestations of Respiratory AL***

Tracheobronchial:	Diffuse parenchymal or alveolar septal:
Nodular (or pseudotumoral):	Dyspnea
Indolent (incidental finding at bronchoscopy)	Physiologic pattern of restrictive lung disease
Bronchial stenosis	Abnormal gas exchange
Diffuse (multiple submucosal plaques):	Recurrent hemoptysis
Segmental bronchial obstruction	Variable radiographic findings (non-specific diffuse interstitial or alveolar opacities)
Dyspnea	Pleural involvement
Hemoptysis	Pulmonary arterial hypertension
Parenchymal nodular:	Respiratory failure due to AL myopathy
Usually asymptomatic	
Must be differentiated from cancer or tuberculosis	

Treatment

Therapy is directed at the underlying plasma cell dyscrasia which is responsible for AL amyloidosis (23,27,32). Alkalating agents such as melphalan, along with prednisone, are the mainstays of this therapy. Results of this treatment are disappointing. However, two major trials which use slightly different regimens of intermittent oral melphalan and prednisone, have confirmed the efficacy of this therapy over no therapy or therapy with colchicine alone (33,34). The median survival of patients in the placebo group was six months and those that received chemotherapy survived for approximately twelve months. Colchicine has been added to the chemotherapy regimen based on the possibility that this drug interferes with fibril formation and not with protein synthesis. There is one clinical study which suggests that Colchicine added to the chemotherapeutic regimen may prolong survival. Aggressive treatment of AL amyloidosis with high dose intravenous melphalan 200 mg/M^2 of body surface area with autologous blood stem cell support as described by Dr. Richard Gaynor in his Grand Rounds, November 6, 1997, may result in the disappearance of monoclonal light chains from the serum, urine and normalization of the plasma cells in bone marrow. Remission of both the plasma cell dyscrasia and clinical symptoms and signs of amyloidosis may occur with this approach, but the duration of the remission remains to be determined. As indicated by Dr. Gaynor, this regimen is highly toxic and not well tolerated by older patients such as those usually afflicted with amyloidosis.

β_2 MICROGLOBULIN AMYLOIDOSIS

β_2 microglobulin is a part of the HLA class 1 histocompatibility complex which is present on the surfaces of all nucleated cells. The heavy chain of the HLA complex is noncovalently linked to the β_2 microglobulin which is an integral part of the cell membrane and thereby attaches the HLA complex to the cell surface. In the early 1980s, it was reported that patients undergoing hemodialysis for chronic renal insufficiency, developed amyloid deposits in the soft tissues of the wrists, giving them a carpal tunnel syndrome. These patients also complained of diffuse arthralgi as primarily involving the shoulder girdle with a decrease in the range of motion (35,36). This disability was present in patients who had been on prolonged hemodialysis and approximately 70 percent of patients dialyzed for ten years or more will have carpal tunnel syndrome and significant limitation of motion in their shoulders, wrists and, often, hip joints. Radiographs showed articular erosion and radiolucent cysts developing within the bone of the shoulders, hips and wrists (35,36). Analysis showed that the amyloid was responsible for this deposition and it was in the joint capsule, synovium subchondral bone and articular cartilage. The amyloid was chemically analyzed and shown to be β_2 microglobulin (37,38).

β_2 microglobulin is present in the plasma as a monomer of 11.8 kDa and most of it is removed by glomerular filtration. Reabsorption of the protein occurs in the proximal tubules where it is normally degraded. Plasma levels are approximately 2 mg/L, but in patients with chronic renal insufficiency, it may be elevated as much as 40-60 fold. This elevation is apparently due to failure of plasma clearance and degradation of the protein that normally circulates (35,39). Treatment of the β_2

microglobulin amyloidosis is supportive. Replacing destroyed joints, particularly the hips, has been successful and stabilizing destructive lesions by orthopaedic surgical procedures has had some limited success. Renal transplantation which reestablishes the normal degradation and clearance of β_2 microglobulin from the plasma has been reported to be successful. There are some recent studies with immunoabsorbant dialysis membranes and other filtration substances that are being studied in an attempt to find materials which will lower the elevated β_2 microglobulinemia.

HEREDITARY AMYLOIDOSES

Hereditary amyloidoses are autosomal dominant diseases and are prototypes of late-onset genetic diseases. An intriguing question is, "How can an abnormal gene, present since birth, produce no apparent damage or symptomatology until ages 40 or older?" Elucidation of the pathophysiology in hereditary amyloidoses may provide important insights that may elucidate other late onset diseases which are of greater frequency in medicine. In hereditary amyloidosis, the amyloid deposits consist of abnormal forms of the plasma proteins. As shown in Table 2, there are many plasma proteins, which when mutated, can form the fibrillar material with characteristics of amyloid.

The Transthyretin Amyloidoses

The most common hereditary amyloidosis is that due to genetic variants of plasma transthyretin. Over fifty mutations of this protein have been described and it is an area of active investigation. Transthyretin was previously called thyroxine-binding-prealbumin. It is a serum transporter of thyroxine and retinol-charged retinol-binding protein. Plasma transthyretin is synthesized by the liver as a single polypeptide chain of 127 amino acid residues (40,41). This protein circulates in the plasma as a tetramer (MW=55,000) composed of four identical monomers (Fig. 5). These tetramers are extremely stable and contain two pockets in which usually a single molecule of thyroxine is bound because of negative cooperativity (42). Four retinol-binding proteins are attached at four different sites as shown in Figure 5 (43). The transthyretin concentration in plasma ranges from 20-40 mg/dl and is decreased at times of acute or chronic inflammation. Transthyretin has been called a negative acute

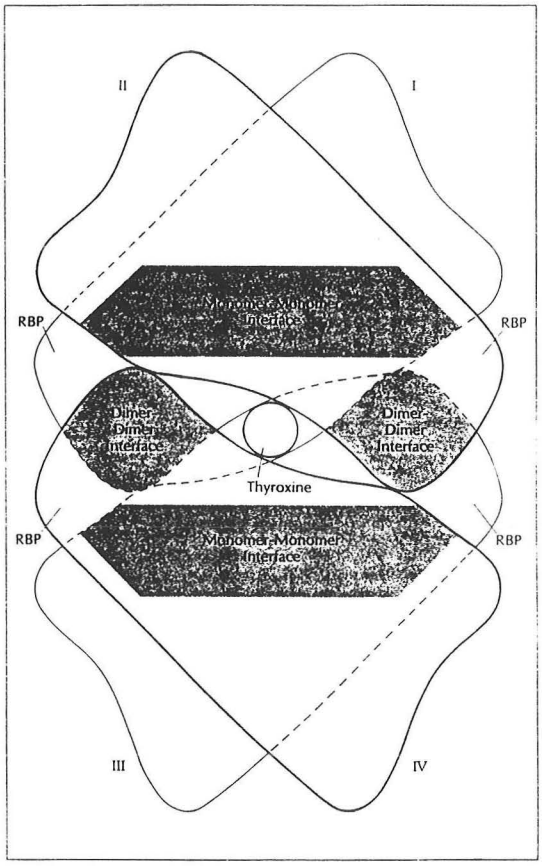

Fig. 5. Two transthyretin monomers immediately join to form dimers, and two of the latter combine into tetramers. The tetramers are extremely stable and contain a "pocket" in which thyroxine is bound and transported. Retinol is also transported, attaching to the tetramers at indicated sites with the aid of retinol-binding protein (RBP). (Adapted from Blake CCF et al).

phase protein because of this depression. It is encoded by a single gene on chromosome 18.

Transthyretin has an extensive β-sheet structure (see Fig. 2). The monomers have eight β chains that are arranged in two sheets. There is a little α helics and a lot of random coil between the β-sheets. The molecules are associated with two major forms of amyloidosis. One is a familial autosomal dominant set of polyneuropathies and cardiomyopathies originally described in Portugal (2), but now known to have a world-wide distribution (1). The Portuguese Familial Polyneuropathy (FAP Type 1) shows the classic and most common features of the hereditary amyloidosis. The mutation is a substitution Met for Val at position 30. The disease usually starts in the third or fourth decade, although in some individuals, symptoms are delayed until old age. The disease progresses over 10-20 years with a peripheral sensorimotor neuropathy, autonomic neuropathy and varying degrees of systemic amyloid involvement. The neuropathy begins in the lower extremities with paresthesias and hypesthesias which can be severely debilitating. Autonomic neuropathy may occur early and patients may present with sexual impotence and gastrointestinal symptomatology. Sensory loss in the lower extremity follows, a stocking distribution as seen in Figure 6. Temperature and pain sensations are impaired earlier than proprioception. When sensory loss has progressed to the level of the knee, the hands usually become involved by sensory neuropathy with a glove-like distribution. Loss of motor strength occurs later and frequently results in foot drop, wrist drop and difficulty in grasp. Trophic ulcers on the lower extremity are common and may produce severe morbidity. Orthostatic hypotension and gastrointestinal dysfunction due to autonomic neuropathy is also common. Involvement of the heart with cardiac amyloid occurs in some patients. Amyloid within the vitreous humor of the eye interferes with vision and may occur in the Portuguese mutation, but is more frequently seen in Swedish kindreds (44). Both the Portuguese and Swedish type mutations are Val 30 Met substitutions although the phenotypes overlap and there are certain tissue patterns of involvement that are different. This may reflect differences in the genetic background for expressing the mutation. Both may have renal amyloid with significant protein loss and subsequent renal insufficiency.

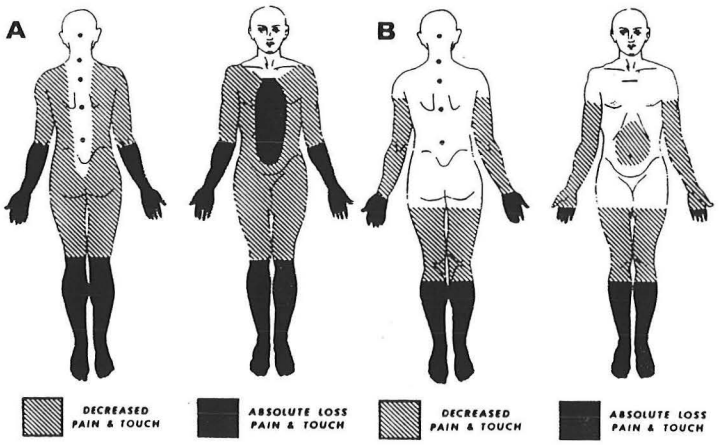

Fig. 6. **A.** Graphic representation of sensory loss in the propositus. Vibratory and position senses were normal at the knee, decreased at the ankle, and absent in the toes. Temperature sensation was intact only in the head and the paravertebral area. **B.** Graphic representation of sensory loss in the brother of the propositus. Vibratory and position senses were absent in the toes but present in the fingers.

Severe cardiomyopathy which is often the cause of death, is seen frequently with the FAP-II mutations which is due to Leu 58 His, Thr 60 Al, or Ile 84 Ser substitutions. However, in the U.S.,

Amyloid). Conduction disturbances occur early and frequently require artificial pacing. The clinical picture is one of a restricted cardiomyopathy with low output heart failure. Cardiomyopathy without a peripheral neuropathy is the main feature of families in Denmark. Approximately half of the 50 transthyretin mutations may be associated with cardiomyopathies.

A description of transthyretin mutations that have been identified in amyloid fibrils or plasma are shown in Table 8. Amino acid substitutions are primarily the result of single nucleotide mutations in the coding regions of the transthyretin gene. The residues involved begin at amino acid residue 10 and occur throughout the coding region to residue 122. There are fifteen mutations in exon 2, nineteen in exon 3, and seven in exon 4. The human gene has four exons and the proximal upstream 5^1- regulatory region has sequences similar to those for the binding of glucocorticoid receptors.

Table 8. ***Transthyretin Amyloidoses***

Mutation	Clinical Name	Clinical Features*	Geographic Kindreds
Cys10Arg		Heart, eye, PN	U.S. (PA)
Pro24Ser		Heart	U.S.
Val30Met	FAP I	PN, AN, eye	Portugal, Japan, Sweden, U.S.
Val30Ala		Heart, AN	U.S.
Val30Leu		PN	Japan
Val30Gly			U.S.
Phe33Ile	Jewish	PN, eye	Israel
Phe33Leu		PN, heart	U.S.
Ala36Pro		Eye, CTS	U.S.
Glu42Gly		PN, AN, heart	Japan
Ala45Thr		Heart	U.S.
Ala45Asp		Heart, PN	U.S.
Gly47Arg		PN, AN	Japan, U.S.
Gly47Ala		Heart, AN	Italy
Gly47Val		CTS, PN, AN, heart	Sri Lanka
Thr49Ala		Heart, CTS	France, Italy
Ser50Arg		AN, PN	Japan
Ser50Ile		Heart, PN, AN	Japan
Ser52Pro		PN, AN, heart, kidney	England
Glu54Gly			
Leu55Pro		Heart, AN, eye	U.S., Taiwan
Leu58His	FAP II	CTS, heart	U.S. (MD)
Leu58Arg		CTS, AN, eye	Japan
Thr60Ala	Appalachian	Heart, CTS	U.S.
Glu61Lys		PN	Japan
Phe64Leu		PN, CTS, heart	U.S., Italy
Ile68Leu		Heart	Germany
Tyr69His		Eye	U.S.
Lys70Asn		Eye, CTS, PN	U.S.
Val71Ala		PN, eye, CTS	France, Majorca
Ser77Tyr		Kidney	U.S. (IL, TX), France
Ile84Ser	FAP II	Heart, CTS, eye	U.S. (IN), Hungary
Ile84Asn		Heart, eye	U.S.
Glu89Gln		PN, heart	Italy
Ala97Gly		Heart, PN	Japan
Ile107Val		Heart, CTS, PN	U.S.
Leu111Met	Danish	Heart	Denmark
Ser112Ile		PN, Heart	Italy
Tyr114Cys		PN, AN, eye	Japan
Tyr114His		CTS	Japan
Val122Ile	Senile cardiac	Heart	U.S.

*AN = autonomic neuropathy; CTS = carpal tunnel syndrome; eye = Vitreous deposits; PN = peripheral neuropathy.

As shown in the Table 8, each of the mutations appear to have a predilection for amyloid fibril formation in selected organs or tissues. However, there is great overlap and amyloid may accumulate in any organ with almost all of the described mutations. The most common mutation is the methionine 30 substitution for valine (FAP I). This variant has been found in many kindreds in Portugal and Japan and also in American kindreds of Swedish, English and Greek origins. It has also been identified in many other countries. In certain areas of Sweden, the gene frequency is as high as three to five percent. It is of interest that homozygous patients with the met 30 gene have a clinical picture very similar to those that are heterozygous.

Senile Amyloidosis

A hereditary form of senile cardiac amyloidosis is caused by the substitution of isoleucine for valine at residue 122. The Val 122 Ile allele is frequent in African-Americans and is present in nearly 4 percent (45). This allele may be responsible for restrictive heart disease and congestive failure in blacks over the age of 60. The diagnosis is most often made postmortem. The mutation has not been detected in Caucasians. However, senile amyloidosis is quite common in other ethnic groups and the protein involved is frequently a normal transthyretin. Senile systemic amyloidosis may be the most common form of systemic amyloidosis occurring in approximately twenty-five percent of people over the age of 80 years. It is generally a benign disorder, although a small percentage of patients develop cardiac amyloidosis with heart failure and/or arrhythmias. However, the clinical consequences of senile massive cardiac infiltration by the transthyretin type of amyloid is relatively less symptomatic when compared with the cardiac disease associated with light chain (AL) amyloidosis. Twelve patients with massive cardiac amyloid infiltration due to transthyretin amyloid, were evaluated (46). These patients were very old, being 82 to 92 years of age and had a mean heart weight of 567 grams (range 310-870). It is of interest that amyloidosis was not suspected in any patient prior to death. Eight of the patients had a history of uncomplicated congestive failure, all of whom responded to standard therapeutic measures. Arrhythmias occurred in all twelve patients with atrial fibrillation in nine, with bundle branch block in three, right bundle branch block in two, and left anterior hemiblock in one. No atrial ventricular dissociation was seen and conduction disturbances were not considered to be the cause of death in any patient. By distinction, cardiac failure and arrhythmias are the leading cause of death in AL amyloidosis and frequently occur in the mutant forms of transthyretin amyloid (47,48).

Aortic amyloid accumulation appears to be the most common form of localized amyloidosis occurring in nearly all people over the age of fifty. There is growing evidence that ninety-seven percent of people over 50 have amyloid accumulation in the media of the aorta. This appears to be benign and there is no associated symptomatology (49).

Treatment of Transthyretin Amyloidosis

There is no specific treatment for the autosomal dominant transthyretin amyloidosis. Most measures are directed at the organ dysfunction. For example, renal dialysis has been used for the severe nephropathy. Cardiac pacemakers have prolonged the life of many individuals with the cardiac

amyloidosis. The restrictive cardiomyopathy of amyloidosis is improved with judicious use of potent diuretics. Because of a concomitant autonomic neuropathy with hypotension, the drug treatment of cardiac amyloidosis with a restrictive pattern presents major challenges to the clinician. Vitrectomy can restore vision to some patients with vitreous opacities, although the effects are often temporary. Plasmapharesis has been tried in some individuals with the transthyretin form of amyloidosis and anecdotal reports suggest some improvement in the quality of life, but no definite therapeutic advantages have been described (1).

Colchicine is commonly given because of its effectiveness in treating the SAA amyloid in Familial Mediterranean Fever. However, there is no evidence that it has any influence on transthyretin amyloid formation, but, nevertheless, it has been given in the hope that it might delay the onset or progression of amyloid formation.

Recently, several patients with transthyretin amyloidosis have received liver transplants (50-52). Since transthyretin is synthesized in the liver, replacement of the organ results in a rapid clearance of the variant transthyretin from plasma. The morbidity from surgery appears to be less than in individuals who have liver transplants for primary liver disease. To date, there is no definitive proof of improved neurological function in individuals receiving liver transplants, although resolution of some bowel dysfunction has been noted in several recipients.

Hereditary Amyloidosis Not Associated With Transthyretin

Table 9 shows the plasma proteins other than transthyretin associated with autosomal dominant systemic amyloidosis. As noted, the clinical features of these rare forms of amyloidosis are primary renal involvement with the nephrotic syndrome and eventually renal failure. Unlike other forms of amyloidosis, the occurrence of hypertension is frequent. There is no apparent relationship between the plasma proteins responsible for these rare forms of amyloidosis, except the fragments that are amyloidogenic have a β-sheet conformation.

Table 9. ***Plasma Proteins (Other Than Transthyretin) Associated with Autosomal Dominant Systemic Amyloidosis***

Protein	Mutation	Clinical Features*	Geographic Kindreds
Apolipoprotein A-I	Gly26Arg	PN, kidney	U.S.
	Leu60Arg	Kidney	England
Gelsolin	Asp187Asn	PN, lattice corneal dystrophy	Finland, U.S., Japan
	Asp187Tyr	PN	Denmark, Czech
Cystatin C	Leu68Glu	Cerebral hemorrhage	Iceland
Fibrinogen	Arg554Leu	Kidney	Mexico
	Glu526Val	Kidney	U.S.
Lysozyme	Ile56Thr	Kidney, skin petechiae	England
	Asp67His	Kidney	England

Apolipoprotein A1 Amyloidosis (Apo A1)

Van Allen and associates described a kindred from Iowa of British descent with an autosomal dominant form of amyloidosis. The patients had prominent renal disease with nephrotic syndrome, hypertension and many developed renal insufficiency (53). Although most of the patients were in their twenties or thirties before manifesting the disease, there have been individuals who lived into their seventies (53,54). Table 10 summarizes the clinical features of hereditary apo A1 amyloidosis and presents the amino acid substitutions responsible for the disease (55). Both normal and mutant forms of apo A1 circulate in the serum. Only the mutant protein is found in the amyloid fibril deposits.

Table 10. ***Clinical Features of Hereditary Apo A1 Amyloidosis***

Kindred (ref) [no. of cases]	Ethnic origin	ApoAI variant	Clinical presentation [age range]	Main clinical features	Outcome
Iowa[41] [14]	British	Gly26Arg	Peripheral neuropathy [26–44]	Neuropathy, peptic ulcer, renal failure, extensive visceral amyloid	Death after 1–12 years
Boston[3] [2]	Scandinavian	Gly26Arg	Hypertension [25–?]	Renal failure, extensive visceral amyloid, no neuropathy	Death after 18 years
Canada[42] [4]	British	Gly26Arg	Hypertension, haematuria [26–46]	Slowly progressive renal failure, asymptomatic visceral amyloid	End-stage renal failure after up to 20 years
Irish* [2]	British	Gly26Arg	Hypertension [38–43]	Progressive renal failure, asymptomatic visceral amyloid	Death or end-stage renal failure after 10 years
English A[4] [5]	British	Leu60Arg	Hypertension, renal failure or organomegaly [25–45]	Progressive renal failure, massive asymptomatic visceral amyloid	All alive up to 12 years from presentation; one case 7 years after renal transplantation
English B* [2]	British	Leu60Arg	Renal and cardiac failure [25–60]	Progressive renal and cardiac failure in daughter, asymptomatic visceral amyloid in father	Heart and renal transplant in daughter; death from stroke at age 64 in father
Jewish [2]	Ashkenazi	Trp50Arg	Haematuria [34–35]	Progressive renal failure, massive visceral amyloid	End-stage renal failure, death after 10 years

*Unpublished.

Gelsolin Amyloidosis

Gelsolin is a protein that binds to, and fragments, actin filaments. It is involved in cell motility and the protein occurs both in the cytoplasm of cells and as a secreted form which is found in leukocytes, platelets, and other cell types. Although the function of the plasma isoform is not known, a major

role may be the clearance of actin from extracellular fluids during injury and inflammation. Several mutations in the gelsolin gene have been described and there are two clinical presentations. One is a peripheral neuropathy accompanied by a lattice corneal dystrophy (56). The second presentation is confined to the cornea (57). There is a network of lattice lines caused by the deposition of an amyloid subunit of mutant gelsolin which is 7-12 kd in size. A variant gelsolin molecule with a substitution of asparagine for aspartic acid at codon 187 of the secreted form of the molecule has recently been described (58). A peripheral neuropathy involving the facial nerve accompanies this amyloid deposition. Cutis laxa with conjunctival involvement is also frequently seen.

Fibrinogen Associated Amyloidosis

Mutations in the fibrinogen Aα-chain gene have been identified in individuals with a familial autosomal dominant form of amyloidosis (59). The disease in these families is relatively early in onset, often appearing before the age of 40 and occurring sometimes in the twenties. The principal manifestation is a nephropathy often presenting with hypertension as well as proteinuria. To date, there is no peripheral neuropathy known to occur with this mutation. It should be noted that a number of mutations in the fibrinogen Aα-chain have been described in individuals with dysfibrinogemia, but none of these has shown an association with amyloidosis.

Lysozyme-Associated Amyloidosis

Lysozyme is a bacteriolytic enzyme present in the plasma and body secretions. It also occurs in polymorphonuclear leukocytes and macrophages. There are two mutations in human lysozyme which have been reported to be associated with a non-neuropathic systemic amyloidosis (60). In both families, the kidneys were involved and in one of the families, there was a petechial skin rash from childhood also present.

Hereditary Cerebral Hemorrhage With Amyloid (Iceland)

This disease becomes apparent in the third or fourth decade of life with the onset of an intracranial hemorrhage. Symptoms depend on the location and severity of the hemorrhage. To date, the reports are confined to Icelandic families (61). Chemical analysis of the amyloid have shown that it is a degradation product of cystatin C (62). Cystatin C is a cysteine proteinase inhibitor which contains 120 amino acids in a single polypeptide chain. It is the product of a single copy gene on chromosome 20.

Hereditary Cerebral Hemorrhage With Amyloid (Dutch)

The clinical picture, again, is dominated by the occurrence of intracerebral hemorrhages, some of

which are fatal, on the first occurrence, and others have recurrent episodes of intracerebral hemorrhage (63). Analysis of the amyloid shows that it is a fragment of the β-amyloid protein precursor which is related to the amyloid found in Alzheimer's disease (64). Senile dementia is not usually a feature of this syndrome, although most of the patients die at an early age from their intracerebral hemorrhage. A mutation at codon 692 with a glutamine substitution for an alanine has been described (64). Individuals in these families which also are of Dutch origin show an early onset form of familial Alzheimer's disease as well as cerebral hemorrhage from the amyloid angiopathy.

Genetic Detection of Hereditary Amyloidosis Carriers

Identification of carriers of genes associated with hereditary amyloidosis is possible when the specific variant is known. The phenotypes of the various hereditary amyloidoses overlap and, therefore, it is essential that the specific protein involved and the mutation be known. It is also difficult to distinguish between the immunoglobulin (AL) amyloidosis and some of the hereditary syndromes because of similarities in the clinical presentation. Since it is usual to treat immunoglobulin amyloidosis with chemotherapy, it is imperative that the hereditary form of the disease be excluded from the diagnosis. Moreover, the prognosis with AL amyloidosis is approximately two years while individuals with the hereditary forms of the disease often live relatively long lives. Since variant forms of plasma proteins are associated with the dominantly inherited amyloidosis, it is possible to detect carriers by isolating and analyzing the plasma proteins that have been implicated in this family's disease (65-67). DNA analysis has been extensively used for detecting variant amyloid associated genes and presents significant advantages when the mutation is known since allele specific oligonucleotides can be prepared, carriers of the abnormal gene are readily detected using a small amount of blood and isolating the DNA (68,69). Many of the disease associated mutations in transthyretin result in new restriction endonuclease recognition sites (70,71). Mutations that result in a loss of a restriction enzyme recognition site can also be detected by this method. These DNA-based tests have allowed the application of molecular biology to the practice of clinical medicine and make the latest methods of genetic counseling and treatment available to individuals with these late onset genetically determined diseases.

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