# Biochemical basis of the amyloid diseases

***Amyloidosis is a heterogeneous group of diseases characterized by normally soluble proteins deposited extracellularly in an abnormally folded, insoluble fibrillar form. This can lead to organ impairment and premature death. This article discusses the pathogenesis, classification system and means of diagnosis of the amyloid diseases.***

Amyloidosis is a heterogeneous group of diseases characterized by whole or fragments of normally soluble proteins deposited extracellularly in an abnormally folded, insoluble fibrillar form. Amyloid deposition is remarkably diverse and can be localized or systemic, acquired or hereditary, rapidly lethal or merely an incidental finding (Goodman and Hawkins, 2005). Clinical consequences occur when accumulation of amyloid fibrils is sufficiently substantial to disrupt the structure and function of tissues and organs, and eventually cause their failure (Pepys, 2001) (*Figure 1*).

There are at least 25 different proteins associated with amyloid fibril formation (Westermark et al, 2005) which, despite heterogeneous structures and functions, can form morphologically indistinguishable amyloid deposits (Sunde and Blake, 1998).

Amyloid fibril formation results from a process of protein unfolding and subsequent refolding and auto-aggregation in a highly ordered β-sheet manner that produces long, rigid and remarkably stable fibrils. The β-sheets consist of strands of polypeptides in zigzag formation (Merlini and Bellotti, 2003). The mature fibril product loosely resembles steel cables with three to six filaments wrapped around one another (Dobson, 2001) (*Figure 2*). Additional non-fibrillar constituents such as serum amyloid P component (SAP) and heparan sulphate are also present in amyloid deposits and contribute to their formation and stability. Electron microscopy reveals rigid, non-branching fibrils around 7.5–10 nm in diameter, and structural studies using X-ray diffraction have shown that all amyloid fibrils share a unique cross-core structure (*Figure 3*). Deposition of amyloid is self-perpetuating once a template of amyloid is present, depending only on a continued supply of the precursor protein. Confirmation of amyloid requires staining of affected tissue with Congo red dye, yielding pathognomonic apple-green birefringence when viewed under cross-polarized light (Goodman and Hawkins, 2005) (*Figures 4a* and *b*).

Amyloid formation is relevant to many different conditions, some of which are quite common. Systemic AL (light chain) amyloidosis is the cause of death of about 1 in 1500 individuals in industrialized countries, while over 1 million people worldwide who are currently receiving haemodialysis are at risk of developing β2 microglobulin amyloidosis. Patients suffering from rheumatoid arthritis, Crohn's disease or inherited fever syndromes are at risk of developing AA amyloidosis. Although the significance of localized cerebral amyloid deposition in Alzheimer's disease and islet cell deposits in type II diabetes mellitus is unclear, both diseases always show histological evidence of amyloid deposition (Hirschfield, 2004).

***Figure 1. Amyloid accumulation in cardiac muscle.***


***Figure 2. Precursor proteins auto-aggregate in a highly ordered abnormal conformation rich in β-sheets to form fibrils.***

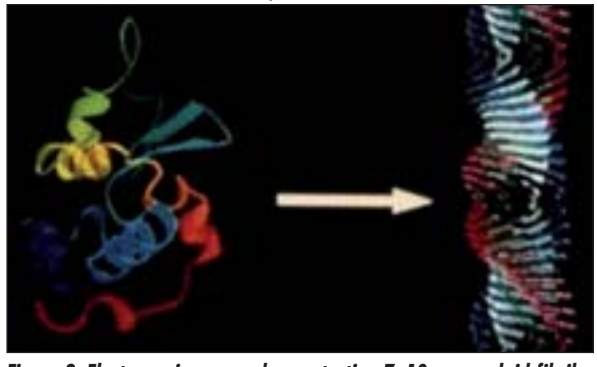
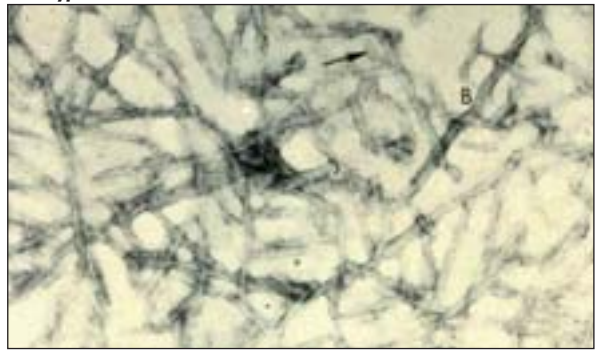


***Figure 3. Electron microscopy demonstrating 7–10 nm amyloid fibrils. B = typical fibril.***

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## Classification

Amyloidosis can be divided into acquired and hereditary forms (*Table 1*).

### Acquired amyloidoses

AL or monoclonal immunoglobulin light chain amyloidosis is associated with characteristically subtle monoclonal gammopathies and is the most common and serious of the systemic amyloidoses. The fibrils are composed of fragments of the monoclonal light chains. Any organ other than the brain may be affected, with the kidneys and heart most commonly involved. The presence of macroglossia, clotting abnormalities or bone marrow deposits is very suggestive of this type of amyloidosis. *Table 2* details the suggested approach to diagnosing and monitoring cases of AL amyloidosis.

AA or amyloid protein A amyloidosis is associated with chronic inflammation and prolonged overproduction of the acute-phase reactant, serum amyloid A protein. The lifetime incidence of AA amyloidosis among patients with disorders such as juvenile and adult rheumatoid arthritis and Crohn's disease is 1–5% after a median latency of about 15–20 years. Renal involvement predominates and, unlike AL amyloid, clinical consequences of AA amyloid are rare in the heart and peripheral nervous system.

β2 microglobulin amyloidosis is a complication of long-term haemodialysis. β2 microglobulin is the invariant chain of cell-surface HLA class I molecules. It is filtered in the glomerulus and exclusively catabolized in the proximal renal tubules. Plasma concentrations subsequently rise in renal failure and dialysis is often inadequate at effective clearance of this protein. β2 microglobulin amyloid deposits become evident after around 5 years of dialysis, with a predilection for bones and joints, although microscopic visceral and vascular deposits do occur and occasionally cause troublesome complications.

***Figure 4. a. Positive Congo red staining of glomeruli indicating amyloid deposition. b. Confirmation of amyloid by apple-green birefringence, viewed under cross-polarized light.***

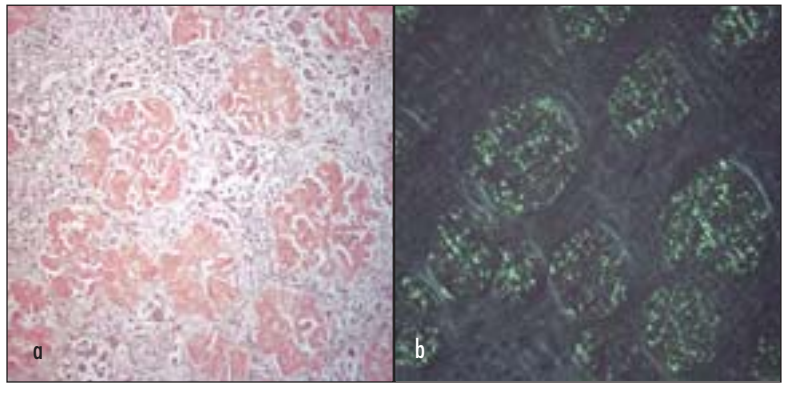



**Table 1. Classification of amyloidosis**

| Type | Fibril precursor protein | Clinical syndrome |
|---|---|---|
| AA | Serum amyloid A protein | Systemic amyloidosis associated with acquired or hereditary chronic inflammatory diseases, formerly known as secondary or reactive amyloidosis |
| AL | Monoclonal immunoglobulin light chains | Systemic amyloidosis associated with myeloma, monoclonal gammopathy, occult B cell dyscrasia, formerly known as primary amyloidosis |
| ATTR | Normal plasma transthyretin | Senile systemic amyloidosis with predominant cardiac involvement |
| ATTR | Genetic variants of transthyretin (e.g. ATTR Met30, Ala60, Ile122) | Familial amyloid polyneuropathy, with systemic amyloidosis and often prominent amyloid cardiomyopathy |
| Aβ2M | β2-microglobulin | Dialysis-related amyloidosis associated with renal failure and long-term dialysis, predominantly musculoskeletal symptoms |
| Aβ | β-protein precursor (and rare genetic variants) | Cerebrovascular and intracerebral plaque amyloid in Alzheimer's disease. Occasional familial cases |
| AApoAI | Genetic variants of apolipoprotein AI (e.g. AApoAI Arg26, Arg60) | Autosomal dominant systemic amyloidosis. Predominantly non-neuropathic with prominent visceral involvement, especially nephropathy. Minor wild-type ApoAI amyloid deposits may occur in the aorta |
| AApoAII | Genetic variants of apolipoprotein AII | Autosomal dominant systemic amyloidosis with predominant renal involvement |
| AFib | Genetic variants of fibrinogen Aα-chain (e.g. AFib Val526) | Autosomal dominant systemic amyloidosis. Non-neuropathic usually with prominent nephropathy |
| ALys | Genetic variants of lysozyme (e.g. ALys His67) | Autosomal dominant systemic amyloidosis. Non-neuropathic with prominent renal and gastrointestinal involvement |
| ACys | Genetic variant of cystatin C (Gln68) | Hereditary cerebral haemorrhage with cerebral and systemic amyloidosis |
| AGel | Genetic variants of gelsolin (e.g. Asn187) | Autosomal dominant systemic amyloidosis. Predominant cranial nerve involvement with lattice corneal dystrophy |
| AIAPP | Islet amyloid polypeptide | Amyloid in islets of Langerhans in type II diabetes mellitus and insulinoma |
| ALECT2 | Leucocyte chemotactic factor 2 | Systemic amyloidosis with predominant renal involvement |

Amyloid composed of peptide hormones, prion protein and unknown proteins not included

Finally in senile systemic amyloidosis, the normal (wild-type) transthyretin protein, or fragments thereof, is the fibril precursor. This form of amyloid is seen in elderly patients (in up to 25% of people over 85 years of age, according to a study by Tanskanen et al (2008)) and predominantly affects the heart. Although commonly asymptomatic, this form of amyloidosis can cause congestive heart failure or heart block.

### Hereditary amyloidoses

Hereditary systemic amyloidosis has lately become better characterized and more widely recognized. In the largest series to date, 10% of 350 patients initially thought to have acquired AL type amyloidosis were subsequently demonstrated to have hereditary types (Lachmann et al, 2002). These are caused by mutations in the coding sequence of a number of proteins, including fibrinogen Aα chain, transthyretin, apolipoprotein AI and AII, lysozyme, cystatin C and gelsolin (Merlini and Bellotti, 2003).

Hereditary amyloidosis has various phenotypes. Familial amyloid polyneuropathy caused by transthyretin variants, the commonest hereditary amyloidoses, usually presents with peripheral and autonomic neuropathy and cardiac disease. Fibrinogen Aα chain, lysozyme and apolipoprotein AI and AII amyloidosis are usually non-neuropathic with prominent renal involvement, while cystatin C amyloidosis presents as cerebral amyloid angiopathy with recurrent cerebral haemorrhage and clinically silent systemic disease (Hirschfield, 2004).

Importantly, acquired AA amyloidosis can also sometimes affect multiple members of the same family, most notably in those with hereditary periodic fever syndromes, and symptoms of the latter are not always evident. These fever syndromes include familial Mediterranean fever, tumour necrosis factor receptor-associated periodic syndrome, hyper-IgD syndrome and the cryopyrin-associated periodic syndromes. Characterization of the specific genetic defects underlying some of these diseases by DNA gene sequencing is vital to determine rational, effective therapies (Hirschfield, 2004).

## Pathogenesis of amyloid

The term amyloid was coined in the mid-19th century to describe starch-like properties of 'waxy' or 'lardaceous' affected organs, and in 1922, amyloid deposits were shown to bind a dye known as Congo red, producing characteristic red staining in normal light and apple-green birefringence when viewed under intense cross-polarized light (Goodman and Hawkins, 2005) (*Figure 3*).

### Protein misfolding leads to fibril formation

Over 20 different proteins can form amyloid in vivo and many more can be made to do so in vitro. Although these proteins have heterogeneous structures, the ultrastructural morphology and histochemical properties of all amyloid deposits are remarkably similar, including their characteristic cross-β secondary structure. Amyloid fibrillogenesis involves marked refolding of proteins and highly ordered self-assembly into protofilaments and subsequently mature fibrils. This very abnormal structure underlies the distinctive physicochemical properties of amyloid fibrils including their relative stability and

**Table 2. Suggested approach to the investigation and monitoring of suspected AL amyloidosis**

| | Confirmation of amyloid | Determination of amyloid type | Evaluation of organ involvement | Investigation of plasma cell dyscrasia | Monitoring |
|---|---|---|---|---|---|
| Pathology | Biopsy and Congo red histology of affected organ, screening tissue (e.g. rectum or fat aspirate) or any available specimen | Immunohistochemical staining of tissue sections with a panel of antibodies to amyloid fibril proteins (often not definitive in AL amyloidosis) | Biopsy of affected organ (but subsequent biopsies merely to determine the extent of amyloid involvement are not recommended) | Bone marrow aspirate and trephine biopsy with immunophenotyping | Follow-up biopsies usually not helpful in monitoring amyloid load |
| Haematology, biochemistry, immunology | | Identification of a monoclonal gammopathy supports AL type, but may be an incidental finding | Serum creatinine and creatinine clearance, 24-h urine protein, liver function tests, coagulation screen, NT-pro BNP and troponin ± thyroid function tests | Full blood count, urea and electrolytes, calcium, creatinine, immunoglobulins, quantitative serum free light chain assay | Quantitative serum free light chain assay (monthly), NT-pro BNP (3–6-monthly), electrophoresis and immunofixation of serum and urine (3–6-monthly) |
| Imaging | SAP scintigraphy | SAP scintigraphy (evidence of marrow involvement is indicative of AL type) | Echocardiogram, electrocardiogram, SAP scintigraphy | Skeletal survey | SAP scintigraphy (6–12-monthly) |
| Other | | DNA analysis for hereditary forms of amyloidosis, amyloid fibril protein sequencing | As otherwise indicated, e.g. nerve conduction studies | | Serial assessment of organ function, e.g. liver and renal function tests, including 24-hour proteinuria estimations, echocardiograms and other investigations as indicated |

NT-pro BNP = N-terminal prohormone brain natriuretic peptide; SAP = serum amyloid P component.

resistance to proteolysis, and their ability to bind molecules of Congo red in a spatially organized manner which results in the pathognomonic tinctoral properties.

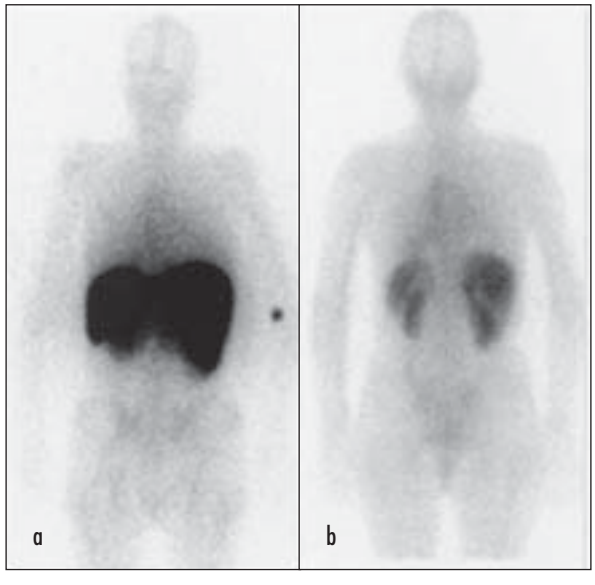
Amyloid deposition occurs under various conditions: first, with the production of an acquired or inherited variant protein with an abnormal structure. For the generation of aggregation-prone and thereby amyloidogenic protein species, modifications may be important, such as mutations, aberrant cleavage or glycosylation. Examples are amyloidogenic monoclonal immunoglobulin light chains in AL amyloidosis, and hereditary amyloidosis associated with variant transthyretin, lysozyme, apolipoprotein AI and AII and fibrinogen Aα chain proteins. Second, amyloid can occur in the presence of a sustained abnormally high concentration of a normal protein, such as serum amyloid A or β2 microglobulin; last, with a normal concentration of a normal, but inherently amyloidogenic protein over a very prolonged period of time, such as seen with transthyretin in senile systemic amyloidosis (Pepys, 2001).

## Other constituents of amyloid deposits

In addition to the major amyloid fibril protein, there are other minor constituents of amyloid deposits. SAP is present in all forms of amyloid, and is bound by the fibrils via a specific calcium-dependent interaction. As a result, radiolabelled SAP has been developed as a nuclear medicine tracer for imaging amyloid deposits, and SAP scintigraphy is used routinely at the National Amyloidosis Centre for diagnosis, assessing extent of disease and monitoring response to therapy (Pepys, 2001; Hawkins, 2002) (*Figure 5*).

Heparan sulphate, in the form of heparan sulphate proteoglycan, is also present in all forms of amyloid and contributes to amyloidogenesis (Pepys, 2001). These molecules can form a scaffold, facilitating the initial phases of fibril nucleation, and may influence the anatomical localization of amyloid deposits in tissue.

***Figure 5. Serum amyloid P component scintigraphy demonstrating (a) extensive amyloid deposition in the spleen, liver and kidneys, which (b) largely regressed after appropriate treatment.***




The role of other molecules in amyloid deposits is less clear. For example, apolipoprotein E is also a common minor constituent of amyloid deposits, and epidemiological studies have shown an increased risk of Alzheimer's disease among white persons carrying the e4 allele of apolipoprotein E. However, the significance of apolipoprotein E in systemic amyloidoses is less clear (Pepys, 2001; Merlini and Bellotti, 2003).

## Factors leading to fibril formation

### Why some light chains are more amyloidogenic than others

Only 2% of monoclonal immunoglobulin light chains are strongly amyloidogenic and perhaps up to about 10% less markedly so. Certain structural features are associated with amyloidogenicity. First, lambda light chains are more commonly associated with amyloid fibril production than kappa with a ratio of approximately 3:1, despite the greater proportion of kappa-expressing plasma cells in normal bone marrow. Also, certain isotypes of light-chain variable regions are more amyloidogenic than others. The variable domains of light chains V(L) mutate during the immune response and some of these physiological mutations affect critical structural sites that result in an aggregation-prone state (Merlini and Bellotti, 2003).

### Amyloidogenic mutations of proteins

In the familial amyloidoses, the substitution of a single amino acid transforms a normal protein into an amyloidogenic one, in particular transthyretin and lysozyme proteins. Transthyretin is a homotetrameric protein with a prominent β-sheet secondary structure, whereas lysozyme consists of a single polypeptide with a predominantly helical structure. Over 100 different mutations in transthyretin have been reported, the majority of which are associated with amyloid fibril production (Suhr et al, 2009). Six pathogenic variants of lysozyme have been reported (Merlini and Bellotti, 2003).

### Decreased stability of proteins

A crucial property shared by genetic amyloidogenic variants is a native conformation that is thermodynamically less stable than that of the normal wild type counterpart. For transthyretin, reduced thermodynamic stability increases the tendency for the tetramer to dissociate into amyloidogenic monomers, and the lysozyme mutations increase the propensity for this protein to exist as partially unfolded highly amyloidogenic forms.

It is important to note that not all genetic variants of these proteins are amyloidogenic. Indeed, the Thr119Met variant of transthyretin has a thermodynamic stabilizing effect on transthyretin tetramers in association with both the wild-type polypeptide (wild type/Met119 genotype) and the most common amyloidogenic variant Val30Met (Merlini and Bellotti, 2003).

**Susceptibility to proteolysis**

In many types of amyloidosis, only a limited portion of the amyloid precursor protein forms the fibril. For example, mutations of gelsolin render it susceptible to proteases, yielding highly amyloidogenic polypeptides. In Alzheimer's disease, the fibrils consist of proteolytic fragments of 39 to 43 residues derived from the 753-residue β-amyloid precursor protein (Merlini and Bellotti, 2003).

**'Natively unfolded' proteins may lead to fibril production**

Some proteins inherently have significant structural plasticity and are known as 'natively unfolded' proteins which favours their interaction with certain ligands and promotes the function of the protein. Such proteins may be susceptible to self-aggregation and, in some cases, amyloid fibril formation. An example is apolipoprotein AI, the structure of which is greatly influenced by its association or otherwise with lipid. The N-terminal domain of apolipoprotein AI is the major constituent of apolipoprotein AI amyloid fibrils. Other lipoproteins, such as apolipoprotein AII, apolipoprotein E and serum amyloid A protein, are all also amyloid-forming proteins (Merlini and Bellotti, 2003).

**Other external factors**

The state of equilibrium outside the cell between fully and partially folded forms of the amyloidogenic variants can vary greatly and is influenced by changes in pH, temperature, oxidation and proteolysis. In addition, local microenvironmental conditions affect the organization of protein deposits. For example, pH influences the processing of immunoglobulin light chains, causing them to form either fibrillar amyloid aggregates or amorphous aggregates associated with light chain deposition disease. The common non-fibrillar constituents of amyloid deposits, such as glycosaminoglycans and SAP, may also hasten the integration of a soluble polypeptide into the stable fibrillar confirmation.

**Tissue specificity of amyloid deposition**

There is extraordinary diversity in the anatomical distribution of amyloid deposits and organ 'tropism' of individual fibril types. Fibrinogen Aα chain amyloidosis occurs predominantly in the kidney, transthyretin Met30 variant usually involves peripheral nerves, and in β2 microglobulin, amyloidosis mainly affects bones and joints.

Truly localized deposition of amyloid also occurs. In localized AL amyloidosis, production of monoclonal immunoglobulin from a very focal clone of plasma cells results in amyloid deposition only in the surrounding area. The site of deposition may depend on the concurrence of several factors favouring the formation of fibrils, such as a high local protein concentration, a low pH, the occurrence of proteolytic processing, and the presence of fibril seeds. Specific interactions with tissue glycosaminoglycans or cell-surface receptors may also be important.

**Mechanism of tissue damage**

There is lively debate about the mechanism by which amyloid causes tissue damage and organ dysfunction. Deposition of large amounts of fibrillary material can subvert the tissue architecture and consequently cause organ dysfunction. Amyloid fibrils may also cause organ dysfunction by interacting with local receptors and can act as a barrier against exchange of metabolites. In transthyretin amyloidosis, soluble oligomeric intermediates of fibril assembly may be cytotoxic in vitro and in vivo (Bucciantini et al, 2002).

## Clinical consequences

Amyloid deposition can be localized or systemic, rapidly lethal or an incidental finding (Goodman and Hawkins, 2005). The symptoms and clinical consequences of systemic amyloidoses depend on which tissues are affected. Lethal complications can occur readily with significant cardiac deposits but amyloid polyneuropathy, vascular infiltration and gastrointestinal manifestations may also cause death. The duration of disease varies between biochemical forms but there is remarkable inter-individual variation.

The natural history of amyloidosis is usually of progressive accumulation, but amyloid deposition is not irreversible. Clinical progression of the amyloid diseases merely reflects that the fibrillar deposits are being laid down more rapidly than they are turning over. Many of the conditions that underlie systemic amyloidosis are typically progressive and unremitting, but regression of amyloid is clearly possible when the underlying inflammatory and other amyloidogenic diseases have been treated successfully.

## Diagnosing and distinguishing different types of amyloidosis

Positive histology for amyloid by Congo red staining is still the gold standard for diagnosis. Rectal biopsy remains an excellent screening method, positive in up to 80% of systemic amyloidosis patients. Biopsies of a clinically involved organ, such as a renal biopsy in the setting of nephrotic syndrome, are positive in 95% of cases. All positive biopsies must be followed up by immunohistochemistry to determine the fibril protein type, often coupled with genetic testing to exclude or confirm hereditary forms. Antibodies for the various amyloid fibril types are widely available. Although immunohistochemistry usually yields definitive results in AA amyloidosis, it is quite often non-diagnostic in AL amyloidosis because of high background staining and failure of antibodies to bind to AL fibrils in some patients.

Histology alone cannot provide information about the overall body load or distribution of amyloid deposits, nor does it permit monitoring of the response to treatment. The National Amyloidosis Centre has developed SAP scintigraphy which not only aids diagnosis, but also gives information about the distribution and load of amyloid deposition in the liver, spleen, kidneys, adrenal glands and bone. Repeat SAP scintigraphy after treatment allows an objective measurement of response to therapy (Pepys, 2001; Hawkins, 2002). Prompt diagnosis of amyloid and

precise confirmation of the fibril subtype are thus vital to facilitate appropriate targeted treatment which can nowadays halt or even allow regression of amyloid in a substantial proportion of patients and prolong survival. BJHM

*Conflict of interest: none.*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## KEY POINTS

- The amyloid diseases are heterogeneous – they can be localized or systemic, acquired or hereditary, rapidly lethal or merely an incidental finding.
- Amyloid fibrillogenesis involves marked refolding of proteins and highly ordered self-assembly into protofilaments and subsequently mature fibrils.
- Over 25 different proteins can be associated with amyloid fibril formation.
- Other components support the fibrillary structure of amyloid deposits such as serum amyloid P component and heparan sulphate. Serum amyloid P component scintigraphy is used to help diagnose and monitor amyloidosis while both serum amyloid P component and heparan sulphate are being investigated as potential targets for therapeutic intervention.
- Confirmation of the type of amyloid – AL, AA, hereditary or senile systemic – using histological and genetic testing, is essential to help predict prognosis and determine appropriate treatment.