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Historical Review

AMYLOIDOSIS: A CONVOLUTED STORY

Amyloid is a substance that appears homogeneous and amorphous under the light microscope and stains pink with haematoxylin-eosin and metachromatically with methyl violet or crystal violet. Amyloid stained with Congo red produces an apple-green birefringence under polarized light. The amorphous hyaline-like appearance of amyloid is misleading because it is a fibrous protein. On electron microscopy, amyloid consists of rigid, linear, non-branching, aggregated fibrils that are 7·5–10·0 nm in width and of indefinite length. The fibrils are insoluble and generally resist proteolytic digestion.

All types of amyloid appear the same with Congo red staining and with electron microscopy. However, the fibrils in primary amyloidosis (AL) consist of the variable portion of a monoclonal light chain (κ or λ); secondary amyloidosis (AA) fibrils consist of protein A, a non-immunoglobulin; familial amyloidosis fibrils are usually composed of mutated transthyretin (prealbumin); senile systemic amyloid fibrils consist of normal transthyretin; and amyloid associated with long-term dialysis consists of β_2-microglobulin.

EARLY CASES

In 1639, Nicolaus Fontanus (Fonteyn; Nicolao Fontano) (Fig 1) reported the autopsy of a young man with ascites, jaundice and epistaxis who had an abscess in the liver and a large spleen filled with white stones. This may have been the first description of the sago spleen of amyloidosis. Thomas Bartholin, discoverer of the lymphatic system in humans, described it in his *Historiarum Anatomicarum Rariorum* (Fig 2). He reported the autopsy of a woman whose spleen was so hard that it could scarcely be cut with a knife. Incision of the spleen produced a sound like that of the cutting of spongy timbers. Both of these autopsy reports were included among the 3000 collected in Theophili Boneti's *Sepulchretum sive Anatomia Practica* published in 1679 (Boneti, 1928) (Fig 3). Boneti was born in Geneva in 1620 and received his medical degree from the University of Bologna in 1643. He retired from practice in 1675 because of deafness, and devoted the rest of his life to the collection of medical knowledge. His *Sepulchretum*, containing 1700 pages, began with a dedication, lengthy preface and an impressive list of authors that began with Hippocrates and extended to the then current time. He divided the cases on the basis of anatomy such as the abdomen, thorax and head. However, he made few comments or deductions and came to no specific conclusions. The book also contains several pages of courteous encomiums from prominent 17th century physicians such as Bartholin and Johann Peyer. Many of his cases represented pulmonary tuberculosis with good clinical histories and convincing post-mortem studies. Forty pages were devoted to neoplasms, and their emphasis was on tumours of bones. He often attributed ascites to diseases of the spleen rather than the liver. Although the *Sepulchretum* is a great collection of cases in the history of pathology, it is marred by the lack of judgement in grouping and separating lesions and the uncritical acceptance of the cited author's opinion.

Wainewright (1722) described a patient who had had 'strumous swellings in his neck' for several years, hepatomegaly (two to three times normal size), and 'a clay-coloured pituitous substance' in the liver, which some believe represented amyloid. F.V. Raspail (1794–1878) froze tissues for microscopic investigation. He also used alcohol to make the tissues more firm and the iodine test to demonstrate starch. Some consider Raspail the founder of histochemistry. J.J. Colin and H.F. Gaultier de Claubry reported in 1814 that starch appeared blue if tested with iodine. Schleiden in 1838 referred to the iodine test for starch. Apparently, Raspail introduced cellular pathology when he stressed the origin of new cells by division. It is not known whether Virchow knew the work of Raspail, the third edition of whose book was published in 1840 just before Virchow's career began (Schwartz, 1970). Abercrombie in 1828 described the liver as a uniform dull yellow that closely resembled the colour of impure beeswax.

LARDACEOUS OR WAXY DEGENERATIONS

Antoine Portal was probably the first to describe substances similar to lard in the liver of an elderly woman in 1789. Portal subsequently described the liver of an 8-year-old boy with scrofula as very large and noted that when exposed to heat it hardened like albumin. He thought that the child had an albuminous obstruction. Lardaceous changes were reported in a letter by Merat in 1818 (Schwartz, 1970). Carl Rokitansky (1842) stated that patients with tuberculosis or syphilis had liver enlargement as a result of infiltration by a grey albuminous, gelatinous substance. He described the firm greyish material as 'lardaceous-gelatinous.' He stated that the lardaceous infiltration occurred in cases of scrofula (tuberculosis), syphilis and mercury poisoning. He thought that the lardaceous or 'waxy' livers were a type of fatty liver. George Budd (1808–1882; Fig 4) described a patient with an enormous liver that appeared pale. In another case, the liver was analysed and consisted of animal matter with

Correspondence: Dr Robert A. Kyle, Mayo Clinic, 200 First Street SW, Rochester, MN 55905, USA. E-mail: kyle.robert@mayo.edu

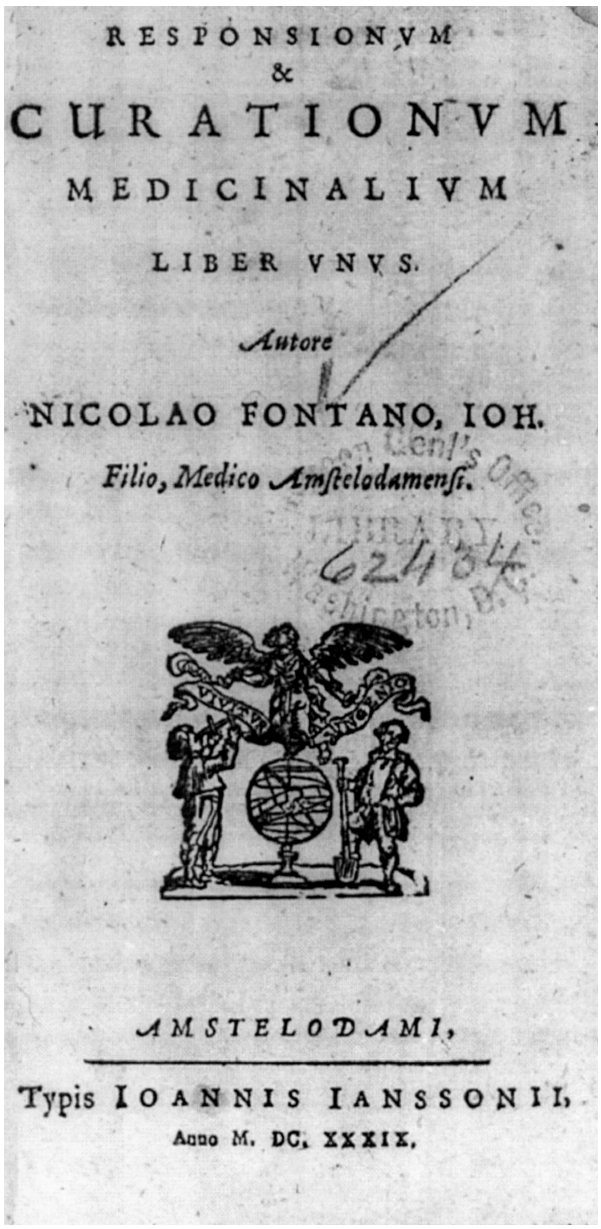
RESPONSIONVM
&
CURATIONVM
MEDICINALIVM
LIBER VNVS.
Autore
NICOLAO FONTANO, IOH.
Filio, Medico Amſtelodamenſi.
AMSTELODAMI,
Typis IOANNIS IANSSONII,
Anno M. DC. XXXIX.

Fig 1. Title page of *Responsionum & Curationum Medicinalium*, Nicolao Fontano.


THOMÆ
BARTHOLINI
HISTORIARUM
ANATOMICARUM
RARIORUM
Centuria I et II.
HAFNIÆ
CIↃ IↃ CLIV.
Typis Academicis Martzani
Sumptibus Petri Haubold Bibl.

Fig 2. *Historiarum Anatomicarum Rariorum*, Thomae Bartholini.

increased albumin (16%) and only 5·75% fat. He concluded that the infiltrating substance was albuminous and not fatty. He also noted that the kidneys of two of his patients showed the same infiltration as in the liver (Budd, 1852). Budd noted that all four of his cases were associated with tuberculosis involving bone.

Gairdner (1854), in his report of the analysis of waxy degeneration of the liver by Dr James Drummond, found that the fat content was not increased above normal. In fact, he stated that the 'waxy organ is unusually poor in oil.' He had compared the fat content of the liver with waxy degeneration with the fat content in cirrhosis, fatty degeneration and normal people. In addition, there was a marked increase in the percentage of solids in the waxy liver. Gairdner concluded that the waxy liver represented a true degeneration in which there was a 'metamorphosis of its glandular structure into a much more dense "albuminous material" than in the normal condition.' He believed that this resulted in a reduction in function and loss of the typical structural characteristics of the liver.

'AMYLOID'

The term 'amyloid' was coined in 1838 by Matthias Schleiden, a German botanist, to describe a normal amylaceous constituent of plants. Rudolph Virchow, in 1854, used the term amyloid because of the peculiar reaction of the corpora amylacea of the nervous system with iodine. He was convinced that cerebral corpora amylacea could be considered identical to starch (Virchow, 1971). He preferred amyloid to the commonly used terms 'lardaceous' or 'waxy' changes. The French school preferred the term

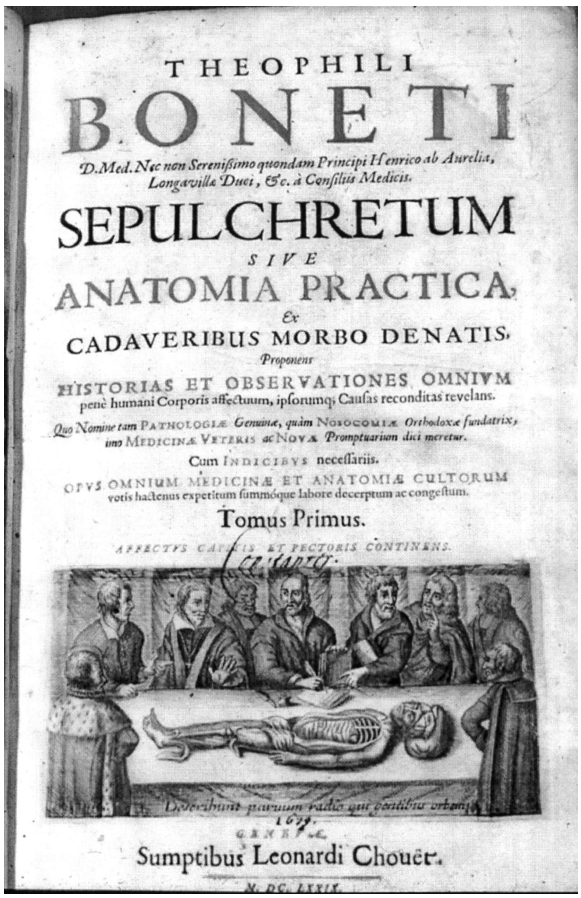
THEOPHILI
BONETI
D. Med. Nec non Serenissimo quondam Principi Henrico ab Aurelia, Longavillæ Duci, &c. à Consiliis Medicis.
SEPULCHRETUM
SIVE
ANATOMIA PRACTICA,
Ex
CADAVERIBUS MORBO DENATIS,
Proponens
HISTORIAS ET OBSERVATIONES OMNIVM
penè humani Corporis affectuum, ipsorumq; Causas reconditas revelans.
Quo Nomine tam PATHOLOGIÆ Genuinæ, quàm NOSOCOMIÆ Orthodoxæ fundatrix, imo MEDICINÆ VETERIS ac NOVÆ Promptuarium dici meretur.
Cum INDICIBVS necessariis.
OPVS OMNIVM MEDICINÆ ET ANATOMIÆ CULTORUM
votis hactenus expetitum summóque labore decerptum ac congestum.
Tomus Primus.
AFFECTVS CAPITIS ET PECTORIS CONTINENS.
Sumptibus Leonardi Chouët.

Fig 3. *Sepulchretum sive Anatomia Practica*, Theophili Boneti.


George Budd (1808-1882)

Fig 4. George Budd 1808–1882. (From Doyle, 1988. By permission of The Royal Society of Medicine.)

'lardaceous' because the tissue looked like bacon, whereas the Edinburgh school preferred the term 'waxy.' They also used the term sagomilz (sago spleen) because its follicles were converted into the waxy changes. They thought that amyloid was more closely aligned with cellulose than starch. Virchow disagreed with Kekule and Schmidt who, like Budd, reported a high proportion of nitrogen in organs infiltrated by amyloid. Virchow thought that analysis of the whole liver was inadequate and stated that 'only when we have discovered the means of isolating the amyloid substance, shall we be able to come to any definite conclusion with regard to its nature.' Virchow believed that the pale organs infiltrated by amyloid resulted from ischaemia from the obstruction of vessels preventing the flow of blood. He pointed out that marasmus was a prominent feature of advanced amyloid disease. He described cases in which the entire digestive tract was involved. Virchow also noted that the iodine test showed reactivity with the glomeruli and the afferent arteries of the kidney.

Johann Meckel noted that the sodium and iodine sulphuric-acid test produced a red or violet colour in many organs considered to be affected by lardaceous degeneration. He emphasized that the lardaceous changes were present not only in the liver and kidneys but also in the aorta, arteries and intestinal wall, which is the distribution of amyloid that is now recognized. Meckel had succeeded Virchow as prosector of the Charite Hospital in Berlin. He believed that the lardaceous deposits consisted of cholesterine. Meckel, who died at age 35, in his post-humous monograph, published by his friend T. Billroth, stated that lardaceous and waxy infiltration was not the result of cellulose degeneration, and that it had no resemblance to starch granules. Schwartz compared Virchow with Columbus, who headed for India and found America, whereas Virchow searched for starch in the human body and discovered amyloid (Schwartz, 1970).

In 1859, Carl Friedreich and August Kekule reported that the waxy spleen contained no material that corresponded chemically to amylon or cellulose. Even though it consisted of albumoid compounds, they believed that the name 'amyloid' could not be changed.

Primary amyloidosis was probably reported in 1856 when Samuel Wilks (Fig 5) described a 52-year-old man with lardaceous viscera in whom the changes were unrelated to syphilis, osteomyelitis, other osseous disease or tuberculosis. The patient had dropsy and albuminuria. At autopsy, the heart was hypertrophied and the spleen was hard and lardaceous. The kidneys were whitish and showed considerable lardaceous changes. However, the patient was atypical in that he had had episodes of dropsy for 8 years and this is much longer than the usual survival of patients

Fig 5. Samuel Wilks 1824–1911. (From Doyle, 1988. By permission of The Royal Society of Medicine.)

with primary amyloidosis and nephrotic syndrome (Wilks, 1856). In 1865, Wilks published an additional 60 cases of lardaceous disease and noted that five of his 96 cases did not have evidence of an underlying disease such as syphilis, tuberculosis or bony disease. He disagreed with the use of the term 'amyloid degeneration' and emphasized its albuminoid nature. He took particular issue with the term 'degeneration' because the liver was filled with adventitious matter and was heavier than normal and, thus, the process was not degenerative. He stated that lardaceous involvement led to destruction of the healthy elements of the liver and kidney. He also mentioned that the term 'waxy cast' had been applied to the wax-like microscopic cast of the uriniferous tubules long before the term 'waxy kidney' came into use. He emphasized that waxy cast and waxy kidney were unrelated (Wilks, 1865). Wilks also reported involvement of the suprarenal (adrenal) glands with lardaceous disease in 1860. The patient had had syphilis and lardaceous disease involving the liver, kidneys and spleen. There was no mention of symptoms related to adrenal insufficiency, except that the patient had general cachexia. The adrenal glands were 'large and remarkably firm' (Wilks, 1860).

A committee of which Wilks was a member was appointed by the Royal Society of London to report 'on the nature of the so-called Lardaceous Disease and as to the name by which it should be recognized.' The committee believed that the tissue contained an increase of cholesterine and was nitrogenous, containing 13–14% nitrogen and, thus, of albuminous origin. However, the committee recommended that the term 'lardaceous' could be adopted by the Society because it was widely used and well understood (Report of the Committee on Lardaceous Diseases, 1871).

Dickinson (1869) presented a case of lardaceous disease of the kidney attributed to an ovarian abscess. He postulated the lardaceous material 'as a precipitation from the blood of fibrine which has been disassociated from the albumin and alkali with which it is normally connected.'

It is not clear why the term amyloidosis prevailed, but the reason was perhaps related to Virchow's standing as a pathologist and to the common use of the iodine stain as a diagnostic test (Doyle, 1988).

The first case of amyloidosis associated with multiple myeloma was reported by Weber (1867). The autopsy revealed spontaneous fractures of the sternum with replacement by a greyish red substance containing many small nucleated cells. Identical findings were present in other bones. The left ventricle of the heart was hypertrophied, and the kidneys and spleen contained amyloid. Adams (1872) described a 60-year-old woman with lardaceous changes in the liver and spleen and multiple pathological fractures. The bone marrow was infiltrated with plasma cells and a pinkish gelatinous material. Despite these reports, many have attributed the first case of primary amyloidosis to Wild (1886). The patient, a 56-year-old woman, had 'weakness of the heart muscle' and died of erysipelas. At autopsy, the ventricles were firm and resistant and contained a hyaline material that stained with iodine, sulphuric acid and methyl violet. Bowel and lung were also involved. The liver, spleen and kidneys were not involved by amyloid. There was no mention of the bone marrow examination.

AMYLOID STAINS AND FIBRIL FORMATION

Aterman (1976) reviewed the metachromatic stains for amyloid. In 1875, André-Victor Cornil of Paris, Richard Heschl of Vienna and Rudolph Jürgens of Berlin independently reported the use of aniline dyes in the recognition of amyloid. The term 'metachromasia' was introduced by William Ackroyd, and Paul Ehrlich used the term 'metachromatic' to describe the staining reaction of amyloid in 1878. All agreed that the metachromatic stains, particularly methyl violet, were much better than the iodine sulphuric acid test. Cornil introduced methyl violet, which allowed him to recognize clearly the extracellular nature of amyloid deposits. It is interesting that Virchow rejected the metachromatic stains for amyloid as long as 10 years after their discovery. The metachromatic stains were eventually replaced by Congo red, which was introduced by Bennhold (1922).

Congo red is an aniline dye that is used for staining textiles. It stains all types of amyloid. The origin of the term is not clear. A major diplomatic conference was held in Berlin in 1884–1885 to mediate a trade dispute between

several European colonial powers concerning the Congo River Basin in central Africa. This conference coincided with the introduction of the new aniline dye. Because the Congo was on the tip of every tongue and represented an exotic place to Europeans, it is not surprising that the dye was given the name 'Congo.' Congo red was used to stain tissue in 1886, but it was not until 1922 that Congo red was found to bind avidly to amyloid (Bennhold, 1922; Steensma, 2001).

Bennhold (1922) noted that an intravenous injection of Congo red in patients with amyloid resulted in disappearance of the dye from the plasma and its accumulation in amyloid tissue. He thought that this could be used for diagnostic purposes because the metachromatic stains and iodine had major shortcomings. He found that frozen sections revealed marked staining of the amyloid with Congo red, but staining of paraffin sections was unsatisfactory. Because both the amyloid and the normal tissue stained, it was difficult to detect the amyloid. Bennhold discovered that exposure of the Congo red-stained tissue to lithium carbonicum in 80% alcohol resulted in staining of only the amyloid. He recommended that paraffin sections stained with 1% watery Congo red, then exposed to lithium carbonicum and destained with 80% alcohol followed by haematoxylin stain resulted in staining only amyloid. He noted that hyaline tissue and corpora amylacea of the prostate did not take up the Congo red stain. He thought that Congo red provided a better definition between amyloid and non-amyloid than the use of methyl violet.

Divry and Florkin (1927) at the University of Liege, Belgium, first described the green birefringence when an amyloid plaque from the brains of patients with Alzheimer's disease exhibited apple-green birefringence when stained with Congo red and viewed under polarized light. Cohen & Calkins (1959) first recognized that all types of amyloid demonstrated a non-branching fibrillar structure when viewed under the electron microscope.

Although each type of amyloid consists of a different fibril protein, its deposits share similar histochemical properties and structural morphology. Amyloid fibrils consisting of amyloid A, λ-light chains, apolipoprotein, and variant lysozymes consist of five or six protofilaments. In contrast, transthyretin amyloid contains four protofilaments. All amyloid fibrils have an electron-lucent core. The structure of amyloid consists of β-sheet folding of the polypeptides within the protofilament (Serpell *et al*, 2000). The sequence of amino acids and the aqueous environment consisting of pH, temperature and ionic strength strongly influence the conformation of the protein (Kelly, 1998). In addition to the fibrillar amyloid protein, heparan sulphate proteoglycan, laminin, collagen IV, serum amyloid P and apolipoprotein E are also components of amyloid deposits. Amyloid P-component is a glycoprotein that is present in all types of amyloidosis. It apparently protects amyloid fibrils from proteolytic degradation, but its physiological function is unknown (Pepys *et al*, 1997). These amyloid deposits may be biologically inert and represent a by-product or 'tombstone' of other pathological processes, or amyloid may be toxic and produce cell damage (Kisilevsky, 2000).

ORIGIN OF PRIMARY (AL) AMYLOID

Magnus-Levy (1931) raised the question of Bence Jones protein as the 'mother substance' of amyloidosis. Seven years later he reviewed 31 cases of multiple myeloma in which amyloid was present in the myeloma or the bone marrow and 18 in which amyloid was present in other organs. He emphasized the occurrence of amyloid in muscles and periarticular areas. He postulated that the myeloma cells produced amyloid but it could not be removed from the area. Subsequently, the accumulated amyloid together with myeloma cells broke through the bone and involved the muscles and fatty tissue. He also noted that amyloid occurred with large or small amounts of Bence Jones protein. He thought that hyperproteinaemia by itself did not produce amyloidosis. He postulated that increased production and decreased metabolism of Bence Jones protein was responsible (Magnus-Levy, 1938). Herbut & Erf (1946) demonstrated the presence of amyloid in plasma cells and believed that this was conclusive evidence of the site of origin of amyloid. Magnus-Levy (1952) predicted that amyloid would be able to be isolated as a pure substance, and that its relationship to other proteins would then be established.

Apitz (1940) also stated that amyloid in the tissues was analogous to the excretion of Bence Jones protein by the kidneys. He emphasized the finding of amyloid in the heart, tongue, periarticular tissues, lung and subcutaneous tissue in multiple myeloma in contrast to its presence in liver, spleen and kidneys in secondary amyloidosis. He also thought there was a chemical difference between the amyloid associated with chronic infection and the para-amyloid associated with Bence Jones protein. The close association of primary amyloidosis with multiple myeloma or a plasma cell proliferative disorder was reported by Kyle & Bayrd (1961). Osserman *et al* (1964) suggested that Bence Jones protein played an important role in primary amyloidosis. Eanes & Glenner (1968) reported that X-ray diffraction studies of amyloid fibrils gave a cross-β X-ray pattern. They interpreted this as a 'pleated sheet' structure formed by the amyloid polypeptide chain folding in a regular manner on itself so that adjacent chain segments were laterally arranged in an antiparallel manner. The axis of the chain segments ran transverse to the axis of the filament axis. Glenner *et al* (1971a) produced purified, reduced and alkylated protein of amyloid fibrils from two patients, and determined the sequence of the NH_2 terminal amino acids. The amino acid sequence of Ker, a Bence Jones κ-protein, was identical except for one of the first 30 residues of one amyloid protein and two of the first 24 positions in the other. This finding suggested that these two amyloid fibrils were portions of a κ-light chain.

It was then shown that monoclonal light chains could form amyloid fibrils. Glenner *et al* (1971b) cleaved three κ- and two λ-Bence Jones proteins from myeloma patients without amyloidosis into their variable and constant

fragments. If the fragments were exposed to pepsin, precipitates formed with the two λ-Bence Jones proteins. These precipitates stained with Congo red and had a green birefringence with polarization microscopy. Electron microscopy revealed fibrils measuring 70–80 Å in width and 1000–2000 Å in length. Examination using X-ray diffraction methods revealed a typical antiparallel β-pleated configuration of amyloid fibrils. These studies demonstrated that amyloid fibrils could be created from Bence Jones protein. The results of peptide mapping, sequence analysis and molecular weight determinations proved that the fibrils were derived solely from the variable portion of the λ-light chain. Because none of the patients whose Bence Jones protein formed amyloid fibrils were known to have amyloidosis, other mechanisms or factors in addition to these are necessary for the production of amyloid fibrils *in vivo* (Glenner *et al*, 1971a).

Solomon *et a*l (1992) demonstrated the presence of amyloid in the kidneys of mice when injected with monoclonal light chains (Bence Jones protein) from two patients with AL amyloidosis. The amyloid deposits were Congo red-positive with green birefringence and had a fibrillar ultrastructure. They consisted of human monoclonal light chains and mouse amyloid P component. The deposition of amyloid was accelerated by dehydration. Mice injected with non-amyloid–associated Bence Jones protein had no or only rare amyloid deposits.

The treatment of AL is unsatisfactory. Therapy with melphalan and prednisone results in longer response rates and prolonged survival compared with colchicine (Kyle *et al*, 1997). Autologous stem cell transplantation is beneficial in appropriately selected patients (Comenzo *et al*, 1998).

FAMILIAL AMYLOIDOSIS

De Bruyn and Stern (1929) described a 52-year-old man who had had pain and numbness of his extremities for 3 years. He had a loss of energy and appetite and then developed severe diarrhoea. Two brothers and a sister had died of a similar illness. At autopsy, microscopic examination revealed masses of a non-nucleated, homogeneous substance in the peripheral nerves. The origin of these masses appeared to be a hypertrophy of the sheaths of Schwann, and they were called 'plasmatic swellings.' Anilin blue-orange G revealed a deep-blue staining of the plasmatic swellings, whereas no staining occurred with the Scharlach R and Weigert-Pal methods. The blood vessels in muscle were slightly thickened. The authors postulated that the non-nucleated masses represented an earlier stage of previously described diseases or a 'slightly different process.' The latter appears correct in that the findings are consistent with familial amyloidosis.

In 1939, Andrade (1952) saw a 37-year-old woman from Povoa de Varzim, near Porto, Portugal, who had a peripheral neuropathy that was known as 'foot disease' in the area. Thirteen years later, he described 74 patients from multiple families who presented with an insidious onset of sensorimotor peripheral neuropathy manifested by paraesthesias or analgesia, muscle weakness and loss of reflexes. The disease began in the second or third decade of life. In addition, abdominal distention, constipation or diarrhoea, loss of sphincter control and reduced libido and potency occurred. Symptoms progressed and death occurred from cachexia or infection within a decade. Biopsy of the nerves revealed amyloid. In 1965, Rune Andersson of Umea University, Sweden, saw a 66-year-old man who had peripheral neuropathy for 15 years. The patient was unable to stand because of severe orthostatic hypotension. Initial biopsy results were negative, but a cousin was found to have peripheral neuropathy with vitreous opacities and this led to a diagnosis of amyloidosis. The first 10 cases from Lappland and Skellefteå were reported in 1968 (Andersson & Kassman, 1968). Andersson (1976) published the findings of 60 patients with amyloidosis and polyneuropathy from northern Sweden. The Swedish patients were similar to those from Portugal except the average age at onset was 55 years and malabsorption and peptic ulcer were more frequent in the Swedish patients.

Residents of Kumamoto and Nagano prefectures in Japan have been reported with familial amyloid polyneuropathy (Tawara *et al*, 1981). The disease usually begins between 25 and 35 years of age, but the onset may occur late in life. The amyloid fibrils of the Portuguese, Japanese and Swedish patients all consisted of transthyretin (prealbumin) (Costa *et al*, 1978). In 1985, Portuguese investigators reported that methionine is substituted for valine at position 30 (TTR Met 30) in the variant transthyretin (Saraiva *et al*, 1985). Liver transplantation has been introduced as a treatment for familial polyneuropathy (Holmgren *et al*, 1993).

It is of interest to find the same mutation (TTR Met 30) in Portugal, Sweden and Japan. The gene may have been transmitted by Vikings from Sweden who visited Western Europe beginning in the 8th century. The greatest prevalence in Portugal is in the region of Povoa de Varzim and Vila do Conde on the coast of Portugal near Porto, and this finding is consistent with this possibility. More than 1000 patients with familial polyneuropathy have been recognized in Portugal. Alternatively, the mutation could have arisen in Portugal and then been carried to Sweden by Portuguese traders who brought salt essential for the preservation of fish and game. There is an illustration of a harbour near Skellefteå showing a merchant from a distant land buying dry fish. Could he be a Portuguese carrier of the gene? Portuguese traders introduced cartography, astronomy, the art of navigation, printing, a new vocabulary and rifles into Japan in the 16th century, and may have been responsible for transmission of the gene (unpublished observations, presented at the First International Symposium on Familial Amyloidotic Polyneuropathy and Other Transthyretin Related Disorders, 1989). This story is part of folklore and is entertaining, but it is not supported by fact. Haplotype analysis suggests strongly that multiple independent mutations occurred at hot spots (Yoshioka *et al*, 1989; Reilly *et al*, 1995).

A family with amyloidosis who were of Swiss origin and residing in Indiana was reported in 1956 (Rukavina *et al*, 1956). The first manifestation is carpal tunnel syndrome followed by peripheral neuropathy involving the lower

extremities. Cardiac involvement is not prominent, and renal involvement does not occur. It is caused by a mutation of transthyretin (SER 84) (Wallace *et al*, 1988).

Familial amyloid cardiopathy was reported in five of 12 siblings in a Danish family with amyloid congestive heart failure in the 4th or 5th decade of life. It led to death in 2–6 years (Frederiksen *et al*, 1962). The amyloid in the heart consisted of transthyretin with a methionine substitution in position 111 (Nordlie *et al*, 1988). An American family from the Appalachian region of the United States was reported with cardiac amyloid from an Ala60 mutation of transthyretin (Benson *et al*, 1987). Often, the patients also had peripheral neuropathy and carpal tunnel syndrome. This mutation has been recognized throughout a wide geographical area. More than 80 mutations of transthyretin have been reported (Connors *et al*, 2000). It is certain that many more mutations will be reported because of improved analytical methods.

Hereditary cerebral haemorrhage with amyloidosis has been recognized in Iceland and the Netherlands. In the Icelandic form, amyloid consisting of a mutant cystatin-C is found in the small arteries, arterioles, meninges and brain (Jensson *et al*, 1987). This has been recently reviewed (Olaffson & Grubb, 2000). In the Dutch form of familial cerebral amyloid angiopathy, repeated cerebral vascular haemorrhages and senile dementia occur. The amyloid is composed of β-protein identical to that found in Alzheimer's disease (Luyendijk *et al*, 1988), except for a glutamine instead of glutamic acid at residue 22.

Type II lattice corneal dystrophy has been reported in southern Finland, and is characterized by cranial neuropathy, leonine facies and systemic amyloidosis (Meretoja, 1969). This autosomal dominant form of systemic amyloidosis is caused by a mutation in the gelsolin gene (Asn187) (de la Chapelle *et al*, 1992).

Nephropathic familial amyloidosis was reported by Ostertag in 1932 (Ostertag, 1950). Patients present with hypertension and mild renal insufficiency that progresses to end-stage renal failure. The amyloid deposits have been shown to have mutations in fibrinogen α-chain (Alu554 and Glu526) (Benson *et al*, 1993; Uemichi *et al*, 1994).

Mutated apolipoprotein A-l may produce neuropathy involving the upper and lower extremities. It begins in the fourth decade of life, and often death occurs as a result of renal failure (Van Allen *et al*, 1969). The fibrils consist of a mutant apolipoprotein A-l with a single-base mutation of arginine for glycine at position 26 (Nichols *et al*, 1988). Three additional apolipoprotein A-l mutations have been reported (Genschel *et al*, 1998). Two families with different mutations of lysozyme have been reported (Pepys *et al*, 1993). The genetics of the amyloidosis was reviewed recently (Buxbaum & Tagoe, 2000).

Several non-steroidal anti-inflammatory drugs and structurally similar compounds have been found to inhibit the formation of transthyretin amyloid fibrils. With use of a structure-based drug-design approach, other specific transthyretin fibril formation inhibitors have been identified (Klabunde *et al*, 2000). Table I summarizes the familial amyloidoses.

Table I. Familial amyloidosis.

Amyloid protein	Precursor	Syndrome or involved tissues
ATTR	Transthyretin	Neuropathy
AapoAl	Apolipoprotein A-l	Neuropathy, nephropathy
AGel	Gelsolin	Cranial neuropathy, corneal lattice dystrophy
ALys	Lysozyme	Nephropathy
AFib	Fibrinogen α-chain	Nephropathy
ACys	Cystatin C	Cerebral haemorrhage

Modified from the International Nomenclature Committee on Amyloidosis (1999). By permission of the Parthenon Publishing Group.

SECONDARY AMYLOIDOSIS

Secondary amyloidosis (AA) has been recognized for centuries. Levin *et al* (1972) reported the amino acid sequence of an acid-soluble fraction that constituted up to 50% of the amyloid fibrils from a patient with familial Mediterranean fever. They also sequenced three other proteins from patients with secondary amyloidosis and reported almost identical findings. This protein did not resemble any known immunoglobulin. It subsequently was designated protein A. The amyloid fibrils of AA consist of protein A, a non-immunoglobulin protein that is a cleavage product of normal SAA (Hermodson *et al*, 1972).

Another type of amyloidosis occurs in familial Mediterranean fever, which is characterized by attacks of fever and pain in the abdomen, chest or joints. It was usually diagnosed as peritonitis and amyloidosis was not recognized (Siegal, 1945). It is found most frequently in non-Ashkenazi Jews and Armenians. More than half of patients have more than one affected family member. Proteinuria, nephrotic syndrome and renal failure occur in the untreated patient (Heller *et al*, 1958). Secondary (AA) amyloidosis often occurs in the absence of treatment. Colchicine is effective in preventing attacks of fever and pain, and will prevent the development of amyloidosis (Goldfinger, 1972). The gene causing familial Mediterranean fever is designated MEFV (MEditerranean FeVer). System mutations have been identified (Livneh *et al*, 1999). Familial Mediterranean fever is also common in Turks and Middle Eastern Arabs.

SENILE SYSTEMIC AMYLOIDOSIS

Senile amyloid deposits have been found in the heart in approximately one-quarter of patients >70 years (Pomerance, 1965). We also have recognized senile cardiac amyloidosis ante mortem. These patients usually present with congestive heart failure, but the median survival is >2 years compared with 6 months in patients with heart failure caused by primary amyloidosis (Gertz & Kyle, 1989). The amyloid deposits consist of normal transthyretin.

DIALYSIS-ASSOCIATED AMYLOIDOSIS

In patients receiving long-term haemodialysis, carpal tunnel syndrome, articular and periarticular pain, and cystic radiolucencies in the bones develop frequently. Amyloid deposition in the synovium, carpal ligament and bone is responsible for the symptoms. The amyloid associated with dialysis consists of β_2-microglobulin (Gejyo *et al*, 1985).

Department of Hematology and Internal Medicine, Mayo Clinic, Rochester, MN, USA

ROBERT A. KYLE

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