Chapter

An Historical Overview of the Amyloidoses

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Abstract

The amyloidoses are a heterogenous group of clinical disorders that share the common finding of the abnormal deposition of insoluble proteins into various organs, with the result that these proteinaceous deposits disrupt cellular function and impair the integrity of the organs involved. Most typically, the abnormal protein deposition is the consequence of abnormal three dimensional folding of the culprit protein. The abnormal folding of the protein, in turn, may be due to a germ line mutation, may be due to an acquired mutation, or may be due to a polymorphism or characteristic of a normal protein that leads to abnormal folding, precipitation, and deposition of the protein, particularly when that protein is expressed at unusually high levels for a prolonged period of time. The clinical manifestations of an amyloid disorder are the consequences of the array of organs involved, the extent of amyloid deposition, and co-morbid conditions present in the individual patient. The array of organs involved, and the extent of organ involvement, in turn, depend in large part on the specific protein that is responsible for the amyloid deposition, and the process driving that protein's production. In this chapter, a chronological overview is intended to summarize the critical insights into the patho-biology of amyloid accumulation of various types. These insights have allowed an improved understanding over time of the of the major subgroups and disease entities of the amyloidoses, leading to some degree of improvement in diagnosis and treatment outcomes. Unfortunately, as of this writing, treatment outcomes still remain poor for a large fraction of patients, and there is need for improvement in all aspects of the evaluation and management of these diseases.

Keywords: amyloidosis, biological subtypes, medical history

1. Introduction

The amyloidoses are a heterogenous group of clinical disorders that share the common finding of the abnormal deposition of insoluble proteins into various organs, with the re sult that these proteinaceous deposits disrupt cellular function and impair the integrity of the organs involved. Most typically, the abnormal protein deposition is the consequence of abnormal three dimensional folding of the culprit protein. The abnormal folding of the protein, in turn, may be due to a germ line mutation - in which case the disease is a hereditary amyloidosis; the abnormal folding may be due to an acquired mutation, for example a mutation resulting in a B cell lymphoproliferative disorder as is seen in AL amyoidosis, also termed

primary amyloidosis - in which an excess of an abnormal immunoglobulin light chain misfolds and results in amyloid deposition; or may be due to a polymorphism in the culprit protein that leads to abnormal folding, then precipitation, and then deposition of the protein, particularly when that protein is expressed at unusually high levels for a prolonged time period, as in the subtype of amyloid diseases termed AA amyloidosis, or as in renal dialysis associated amyloidosis. The clinical manifestations of an amyloid disorder are the consequences of the array of organs involved, the extent of amyloid deposition, and co-morbid conditions present in the individual patient. The array of organs involved, and the extent of organ involvement, in turn, depend in large part on the specific protein that is responsible for the amyloid deposition, and the process driving that protein's production.

In this chapter, a chronological overview is intended to summarize the critical insights, over time, into the patho-biology of amyloid accumulation of various subtypes. These insights have allowed an improved understanding over time of the of the major subgroups and disease entities of the amyloidoses, leading to some degree of improvement in diagnosis and treatment outcomes. Unfortunately, as of this writing, treatment outcomes still remain poor for a large fraction of patients, and there is need for improvement in all aspects of the evaluation and management of these diseases.

2. Early observations

Robert Kyle, of the Mayo Clinic, a leading investigator in the field of amyloidosis over much of the past century, meticulously detailed early observations regarding cases suggestive of amyloid diseases documented in the medical literature, in a historical review that he published shortly after the turn of this century [1]. Kyle references reports by Theophili Bonetti, in Bonetti's work *Sepulchretum sive Anatomia Practica*, which is included in E. R. Long's book A History of Pathology [2]. Bonetti's reports includes descriptions of two autopsies with findings suggestive of amyloidosis. The earliest of these reports is that of Nicklaus Fontanus, from the year 1639, whose report was of an autopsy of a young man who had evidence of epistaxis, jaundice, and ascities, with gross pathology showing abnormalities of the liver and spleen. Kyle also cites the book by Schwartz [3] to note an autopsy report from the year 1818, by an investigator named Merat, who described "lardaceous" changes in the liver - that is, changes in the appearance of the liver that appeared to show infiltration of the liver by a substance resembling lard - porcine fat. Subsequently, George Budd, in 1852, described several patients with liver infiltration by an abnormal substance, and in his chemical analysis, using the techniques available at the time, he reported these contained a significant amount of albumin - that is, protein, measured at sixteen percent of the infiltrate, with only approximately six percent fat, despite its appearance. Two of the subjects in his series showed similar infiltration of the kidneys, a pattern consistent with current presentations of amyloidosis [4]. In 1814, Colin and Gaultier de Chaubry observed the blue color change seen when starch is stained using iodine together with sulfuric acid. The pathologist Rudolph Virchow applied the term "amyloid" in the year 1854 to characterize the brain structures corpora amylacea due to the color changes seen with the application of iodine, expressing the belief that there was a starch like substance present [5]. In 1842, Carl Rokitansky reported hepatomegaly with hepatic infiltration by a gelatinous material, in a series of autopsies of patients with tuberculosis or syphilis. This may have been the first report of AA amyloidosis in the setting of chronic inflammation and infection. Similarly, in the year 1867, H. Weber reported amyloidosis in a patient with myeloma, with amyloid having been identified in the heart, kidneys, and spleen, consistent with what is now termed AL amyloidosis [6].

In the late nineteenth century, dyes were being widely explored for use in a variety of biochemical investigations, including for use as histopathologic stains. The aniline dye Congo Red was developed in 1883, when Paul Böttinger, at the Bayer Company in Germany, synthesized the compound as a potential pH indicator [7]. In 1923, Bennhold reported administration of Congo Red by intravenous injection into humans [8]. He injected solutions of Congo Red into twenty-one healthy subjects, and into twenty-one patients with a variety of illnesses, including patients with amyloidosis. He noted that in patients with amyloidosis, Congo Red cleared from the blood significant faster than in healthy individuals or patients with other disease states. In that report, one patient with a diagnosis of amyloidosis died within a day of the injection of Congo Red; at autopsy, the liver and spleen appeared to have been stained red by the injected dye Congo Red. In addition, light microscopy of histopathologic slides demonstrated red staining. The observation that a so-called "apple-green birefringence" could be observed in tissue involved by amyloid deposition under polarized light microscopy was made in the mid-twentieth century, variously attributed to Missmahl, a student of Bennhold, and to Divry and Florkin [9]. Over the course of the twentieth century, the technique of Congo Red staining has been refined, and the chemical basis of the staining has been generally well characterized. In an excellent review, Yakupova and colleagues detail both the empiric data, as well as biochemical models, regarding Congo Red staining of amyloid, in the context of the knowledge of amyloid structure [10]. They detail the pitfalls involved in the use of Congo Red staining as a histopathologic test for amyloidosis, and summarize the literature, including a discussion of false positives and false negatives with regard to the accurate diagnosis of amyloidosis.

As X-ray diffraction became available as a technique to study submicroscopic structure, this technology was applied to the study of amyloid. Eanes and Glenner reported X-ray diffraction studies on amyloid filaments in the year 1968 [11], from which it appeared that amyloid is composed of polypeptide chains in a "cross β conformation". Less than a decade prior to that, Cohen and Calkins applied electron microscopy imaging to recognize that all subtypes of amyloid exhibit a non-branching fibrillary structure [12]. Bonar and colleagues refined the characterization of amyloid fibrils using X-ray diffraction to show that for amyloid fibrils, the cross β proteins - polypeptide chains that form β pleated sheets - the individual strands of each β sheet run perpendicular to the fibril axis, with 4.1 Å spacing [13]. Thus, by the 1960s, the general structure of amyloid had been defined, although the underlying pathophysiology of the various subtypes remained to be more completely elucidated.

In recent decades, the application of high performance liquid chromatography (HPLC) and mass spectroscopy (MS) to the analysis of amyloid specimens has greatly enhanced the ability to diagnosis patients as having specific subtypes of amyloidosis, and thus has allowed marked improvements in disease-directed treatments. Mass spectroscopy is an analytical technique that is used to measure the mass-to-charge ratio of ions. The results are typically presented as a mass spectrum, a plot of intensity as a function of the mass-to-charge ratio. Comparison of the results of analysis of a specimen to know materials assists in identification of the composition of the specimen. The scientific basis for the technology of mass spectroscopy began with the work of Eugene Goldstein and Wilhelm Wein in Germany at the end of the 19th century. It was developed into a practical tool by J.J. Thompson in England, and refined by Arthur Jeffrey Dempster and F.W. Aston in the early 20th century. Similarly, HPLC a technique in analytical chemistry used to separate, identify, and quantify each component in a sample that is presumed to be a mixture. The device pumps a highly pressurized liquid solvent containing the sample mixture through a column filled with a solid adsorbent material, and

the components are thereby separated and may be identified by comparisons to know controls. Although conventional liquid chromatography is a technique that was developed much earlier, modern high pressure liquid chromatography matured in the 1960s and 1970s. Thus, the application of this technology to characterize amyloid deposits only began in the 1970s, as discussed later, regarding the work of Mark Pepys and colleagues in characterizing the amyloid P protein.

3. Insights into patho-biological subtypes and diagnosis

3.1 AA amyloidosis

As noted above, autopsy studies in the nineteenth century, as exemplified by the report by Rokitansky in 1842, mentioned above, established a relationship between chronic inflammatory diseases - such as tuberculosis and syphillis, and the development, in some of these cases, of amyloidosis. Earl P. Benditt, a pioneer in the field of amyloidosis, together with his colleague Nils Eriksen at the University of Washington, isolated, by gel electrophoresis, a protein that he initially named "amyloid of unknown origin" in the year 1961 [14], obtained from specimens of patients with "secondary" amyloidosis - that is, in patients with underlying chronic inflammatory diseases. A decade later, in 1971, Benditt and colleagues refined their identification of the amino acid structure of secondary amyloid, showing a specific sequence of amino acids at the N-terminus [15]. The following year, in 1972, Levin and colleagues reported the complete seventy-six amino acid protein sequence of an amyloid protein from a subject with secondary amyloidosis [16]. A number of other laboratories identified similar peptide sequences from specimens of secondary amyloidisis, differing slightly in length, but all sharing the same N-terminal sequence, and with lengths on the order of approximately seventy-six amino acids.

These then became known as AA amyloid, or amyloid A protein. In 1975, Linke and colleagues, using antibodies raised against amyloid A proteins, identified a serum substance that bound these antibodies. Ultimately, this 104 amino acid peptide was identified as the serum precursor to tissue amyloid A, and was named serum amyloid A, or, SAA. There is now a large body of literature detailing the biology of SAA; the SAA proteins are acute phase reactants, which are important both in lipid transport and in inflammation, and participate in the interactions between lipids and the inflammatory process. These molecules are also termed apolipoproteins, in view of the function that they fulfill in transporting lipids throughout the body. The genes for human serum amyloid A1 (SAA1) and human serum amyloid A2 protein (SAA2) map to the region of chromosome 11 p15.1, and are rapidly synthesized by hepatocytes and secreted into the blood in response to a variety of inflammatory stimuli. In experimental conditions, exposure to lipopolysaccharide from *streptococcus pneumonia* provokes a dramatic rise in SAA levels and is associated with chemotaxis of leukocytes as well as a cascade of other inflammatory responses. SAA3 appears to be a pseudo-gene, with no significant protein expression, and SAA4 is expressed constitutively in the liver. AA amyloid fibrils include deposition of SAA1 and. SAA2, and this typically occurs when a significant inflammatory process persists over a long period of time. An excellent recent review of the literature on the structure and biology of serum amyloid A was published by George Sach in the journal Molecular Medicine [17].

In parallel with studies that defined the identity and structure of the amyloid A proteins, during the 1960s a number of investigators identified a separate protein extracted from amyloid deposits, by exploiting antigenic properties, using antibodies to isolate and characterize this protein. Prominent among these investigators

were Cathcart and Cohen, and their colleagues at Harvard Medical School [18]. In a series of experiments reported over several years, this protein was defined, and named amyloid P protein [19], also called in the literature amyloid P component. In the early 1970s, Mark Pepys in London developed a rabbit antibody to C reactive protein (CRP) that also precipitated second protein from serum; this proved to be serum amyloid P (SAP). Over the next two decades, it was discerned that both CRP and SAP are members of the same class, pentameric molecules that have been named pentraxins [20]. Pepys and colleagues reported the three dimensional structure of human SAP using X-ray crystallography in 1994, ultimately finding that this forms a flattened β "jelly roll" structure [21]. The gene for SAP in humans resides on chromosome 1 at locus 1q23.2. Human SAP avidly binds to chromatin, displacing H1 histones; this has suggested that SAP may play a role in modulating DNA biology [22]; however much remains to be determined in this regard. An evolving literature indicates that the so-called "short pentraxins", which include CRP and SAP, are participants in the innate immune response. There is data that both CRP and SAP interact with pathogens to activate leukocytes, as well as to regulate complement; this has been carefully reviewed in a paper by Cox, Pilling, and Gomer of the University of Texas [23]. On average, approximately fourteen percent of the mass of amyloid deposits are amyloid P protein, across amyloid subtypes. In 1979, Pepys and colleagues demonstrated that, in vitro, amyloid P protein binds to both primary amyloid (immunoglobulin light chain amyloid), or AL amyloid, as noted above, as well as binding to secondary amyloid, such as AA amyloid. The amyloid P component is thought to stabilize other fibrillary molecules within the amyloid, such as the amyloid A protein, and inhibit fibril breakdown. Analysis of amyloid deposits by high performance liquid chromatography and mass spectroscopy has become an important tool in confirming the specific subtype of amyloidosis in a patient; a biopsy specimen processed by laser micro dissection can generally be sent to a reference laboratory for such analysis [24]. Such analysis permits determination and confirmation of the specific subtype of amyoidosis; e.g., that a particular specimen contains AA amyloid, AL amyloid, ATTR amyloid, or a less common subtype among the amyloidoses. This mass spectroscopy and high performance chromatography analysis has demonstrated the presence of other moieties present in amyloid deposits, prominently including glycos-aminoglycans, in addition to the ubiquitous amyloid P protein.

In the late 1980s, Pepys and colleagues developed the diagnostic technique of radioactive iodine labeled SAP scintigraphy for diagnosis and evaluation of patients with various forms of amyloidosis [25]. This diagnostic modality remains an important tool to this day in the assessment of patients with suspected to documented amyloidosis, including for the purpose of monitoring response to therapy.

In 2007, researchers in the United Kingdom published the findings of a longitudinal study of 374 patients with AA amyloidosis followed at the Royal Free hospital [26]. They reported that median survival from diagnosis was 133 months, and kidney dysfunction was the predominant clinical manifestation. They further reported that mortality and renal impairment correlated positively with SAA serum concentrations. In this series, underlying chronic inflammatory disorders included chronic inflammatory arthritis most commonly - predominantly Rheumatoid arthritis, chronic infections including bronchiectasis, infections in the setting of chronic injection drug abuse, and infectious complications of paraplegia. Less common underlying inflammatory processes included osteomyelitis, tuberculosis, and periodic fever syndromes such as familial Mediterranean fever; Crohn's disease, and Castleman's disease. Successful management of the underlying inflammatory disorder was associated with improved outcome and lower levels of SAA in the blood.

3.2 AL amyloidosis

Robert Kyle of the Mayo Clinic has published several historical reviews of the disease multiple myeloma. In a manuscript detailing medical observations and insights regarding myeloma, Kyle and Rajkumar [27] cite a case reported in the year 1844, of a 39 year old woman who experienced fatigue and bone pain associated with multiple fractures [28]. The patient died approximately four years after her initial presentation, and autopsy findings included marrow replacement by aberrant cells. A landmark case was that of the patient Thomas McBean, who also developed fatigue and bone pain. Urine specimens from Mr. McBean were brought to Henry Bence Jones, a chemical pathologist, after the patient's attending physician noted a high specific gravity to the urine, as well as opacity of the urine when boiled. Bence Jones reported the findings of proteinuria [29, 30], although he considered that the protein was an oxidized albumin. Waldeyer was the first to use the term plasma cell to describe a specific cell type [31]; however, it was Wright who felt that the malignant cells of myeloma were plasma cells [32]. H. Weber, in 1867, reported an autopsy with findings of non-traumatic fractures of the sternum, with the marrow replaced by an infiltrate of small uncleared cells. The heart was hypertrophied, and amyloid was identified in both the kidneys and the spleen - a presentation consistent with myeloma complicated by amyloidosis [33]. Kyle writes that this was the first report of amyloidosis associated with multiple myeloma. During the first half of the twentieth century, technology evolved to permit the identification of different classes of Bence Jones proteins, and in 1962, Edelman and Gally showed that serum monoclonal light chains from a patient with IgG myeloma shared the same amino acid sequence as the patient's Bence Jones protein, establishing that Bence Jones protein is derived from clonal paraprotein [34].

Magnus-Levy, who began his career in Germany, but relocated to the United States early during the Second World War, documented his conjecture that Bence Jones protein excreted in the urine of myeloma patients might be "the mother substance" of amyloidosis; this was published in the year 1931 [35]. In 1946, Herbut and Erf showed that amyloid could be identified within plasma cells, and concluded corrrectly that plasma cells were the source of amyloid in the setting of myeloma [36]. Thus, by the mid-twentieth century, it was evident that one form of amyloidosis was the consequence of a clonal plasma cell disorder. Glenner and colleagues demonstrated *in vitro* that monoclonal immunoglobulin light chains from myeloma patients could form amyloid fibrils under experimental conditions, which precipitated after pepsin exposure; the precipitates stained with Congo Red, and demonstrated green birefringence by polarizing microscopy [37]. In the past several decades, it has been shown that specific clonal light chain sequences result in a significant predisposition to misfold, and therefore deposit as amyloid; among these, the light chain variable region sequences Vλ1, Vλ2, Vλ3, Vλ6, and Vκ1 are particularly over-represented as amyloid protein, as compared to other immunoglobulin variable region sequences [38].

The treatment of primary, or, AL amyloidosis remains unsatisfactory. This is, in part, due to a frequent delay in diagnosis, which may result either from the lack of specificity in early symptoms, or due to presentation when there is already significant end-organ damage that precludes aggressive therapy [39]. Treatment of all subtypes of amyloidosis are directed, at least in part, to suppressing production of the misfolding protein. In AL amyloidosis, this means suppressing production of clonal light chains secreted by the clonal plasma cells. In 1958, Blokhin and colleagues reported benefit from treatment using melphalan in a small cohort of patients [40]. Similarly, in 1962, Maas reported a study in myeloma patients treated using prednisone, versus placebo, and documented the at least transient objective

anti-neoplastic activity of corticosteroid therapy in treating myeloma [41]. In 1969, Alexanian and colleagues published a seminal prospective, randomized clinical trial that established the combination of melphalan and prednisone as the standard of care as systemic anti-neoplastic therapy for multiple myeloma [42]. That standard prevailed for several decades, until the turn of this century, when novel agents, including the imids and subsequently the proteosome inhibitors, were developed. Consequently, in view of the ability of theses drugs to suppress the malignant clone of plasma cells in overt myeloma, the same drugs were employed to suppress the clonal plasma cell population producing amyloid in AL amyloidosis. Toward the end of the twentieth century, colchicine was used as therapy for AL amyloidosis. However, a prospective, randomized clinical trial comparing colchicine to melphalan plus prednisone, or all three drugs together, in patients with AL amyloidosis, showed a survival advantage from melphalan and prednisone [43]. This was therefore conventional therapy for AL amyloidosis for several decades, until the advent of the newer agents used to treat multiple myeloma - the imids and proteosome inhibitors, and the demonstration that a favorable outcome - as compared to historical controls - could be obtained in carefully selected patients treated using high dose melphalan with autologous hematopoietic rescue. To date, there have been no large, prospective randomized clinical trials in AL amyloidosis of the newer agents, nor of autologous transplants in AL amyloidosis. However, based on numerous Phase II trials, a general consensus has evolved, with patients deemed fit taken to autologous transplant, often after induction therapy. One example of such a consensus approach is an algorithm for treatment published by the Swiss Amyloidosis Network [44]. This group currently recommends induction therapy using the combination of cyclophosphamide, bortezomib, and dexamethasone ("CyBorD") followed by high dose melphalan with autologous hematopoietic rescue, for transplant-eligible patients. For transplant ineligible patients, this group recommends the monoclonal antibody daratumumab, directed against the plasma cell surface protein CD 38, either alone or with combination with other agents. Current investigational therapeutic approaches include novel experimental treatments, such as a monoclonal antibody that can bind and potentially extract amyloid P protein from organ deposits [45].

3.3 ATTR amyloidosis

In 1952, a neurologist, Andrade, reported detailed observations regarding a cluster of patients with neurological deficits in the Oporto region of Portugal, with some of those observations dating back to 1939 [46]. The afflicted patients shared clinical features of peripheral motor weakness and peripheral sensory deficits, as well as, in many cases, gastrointestinal and sexual dysfunction. Histopathologic examination revealed amyloid deposition, and a familial, autosomal dominant pattern of inheritance was noted. Similar observations were made of familial amyloidosis with primarily neurological manifestations - most prominently peripheral neuropathy and autonomic neuropathy - in Japan, and in Scandinavia in the latter part of the twentieth century. Eventually, hereditary amyloidosis was identified in many populations, with clinical syndromes that presented primarily as neurological dysfunction, or with clinical syndromes that presented primarily with cardiac disease. Abnormal electrophoretic mobility transthyretin was noted in many of these cases. With the advent of the technologies of molecular biology of the gene, the vast majority of hereditary amyloidosis cases were found to be due to a variety of mutations in the protein transthyretin became recognized. Hereditary transthyretin amyloidosis, also termed ATTRv amyloidosis ("ATTR variant") is generally an autosomal dominantly inherited disorder, due to a variety of mutations in the

TTR gene that encodes for transthyretin. The TTR gene product is a homodimeric plasma protein produced primarily in the liver, with additional production and secretion by the choroid plexus and by retinal epithelia. The TTR protein serves both as a thyroid hormone binding protein, as well as a retinol binding protein, and was originally called "pre-albumin" due to its migratory pattern on protein gel electrophoresis, running ahead of the albumin peak [47]. Beginning in the late 1980s into the early 1990s, DNA sequencing of the TTR gene by many investigators uncovered numerous mutations in TTR associated with amyloidosis, manifesting as predominantly either neurological disease, or as cardiac disease- typically with arrhythmia, with or without heart failure syndrome [48]. As of this writing, more than 125 different TTR mutations have been identified in the TTR gene locus, that are associated with ATTR amyloidosis. The most common TTR variants in the United States include (1) the Val30Met mutation, which is the most commonly identified mutation worldwide. The syndrome Familial amyloid polyneuropathy (termed FAP) is most commonly caused by Val30Met. Other relatively common driver mutations include (2) the Thr60Ala mutation, (3) the Leu58His mutation, (4) the Ser77Tyr mutation, and (5) the Val122Ile mutation. This last mutation is predominantly seen in the African-American population, and typically presents as cardiomyopathy. The syndrome Familial Amyloid Cardiomyopathy (FAC) is commonly caused by Val122lle.

During the same time period, it was recognized, primarily from autopsy studies, that there were individuals who were found to have evidence of systemic amyloid deposition at an advanced age, primarily in the heart, but in some cases affecting the peripheral nerves, and this has been termed Systemic Senile Amyloidosis (SSA). Often there is no clinical heart disease in these individuals, but some of these individuals will indeed develop cardiac disease, ranging from arrhythmia to heart failure syndrome. There is also increasing evidence that ATTRwt disease is a cause of carpel tunnel syndrome in the elderly. Analysis of the amyloid from these patients showed transthyretin as the major component of the amyloid, together with serum amyloid A protein; there is also data to suggest that the molecule Clusterin may play a role in the formation of this form of amyloid [49]. Clusterin, also called apolipoprotein J, is a heterodimeric protein member of the heat shock protein family, and participates in apoptosis. Critically, gene sequencing of the TTR in these individuals is normal, as was first documented in a study by Westermark and colleagues in 1990 [50]. This subtype of ATTR amyloidosis is also termed ATTRwt, that is, wild-type ATTR Amyloidosis. In this disorder, there is misfolding of transthyretin, despite the normal gene sequence.

When ATTR amyloidosis is suspected due to clinical findings, a tissue diagnosis of amyloid deposition is a definitive procedure. The finding of amyloid deposition, however, should be followed by biochemical analysis of the amyloid, either by mass spectroscopy and high performance chromatography, or by sequencing of the TTR gene, or both. As noted above, ATTRwt is defined in part by a normal gene sequence of TTR. As discussed previously, as in all subtypes of systemic amyloidosis, nuclear medicine studies may be diagnostic [25, op cit]. Myocardial radiotracer uptake at bone scintigraphy using an agent such as technicium-99 pyrophosphate is both sensitive and specific for a diagnosis of cardiac amyloid due to ATTR amyloid, once monoclonal light chain amyloid has been excluded by immunological studies. In the management of ATTRv amyloidosis, liver transplant became a standard of care over the past 25 years, as this replaces the source of the variant, or, mutated TTR with a liver that produces normal TTR. Liver transplant has been documented to improve overall survival in ATTRv amylodosis [51]. In ATTRv amyloidosis, the mutations in the TTR gene appear to destabilize the normal tetrameric state of the transthyretin protein, resulting in dissociation into monomers prone to misfolding

and aggregating. Consequently, it was hypothesized that agents that could stabilize the TTR tetramers might ameliorate the disease by reducing amyloid fibril formation and deposition. Tafamidis is an oral agent, currently approved in Europe for the treatment of early stage polyneuropathy ATTR amyloidosis, which appears to work by stabilizing the transthyretin tetramer. In prospective, randomized clinical trials, Tafamidis was shown to be safe, with evidence of that it retards neurological deterioration [52, 53]; however efficacy was questionable for patients with advanced disease. Subsequently, a prospective, randomized, placebo controlled trial of Tafamidis in ATTR cardiomyopathy, both variant and wild type, NCT01994889, showed a thirty percent reduction in mortality as compared to placebo [54]. This led to FDA approval in the United States of Tafamidis for the treatment of ATTR cardiomyopathy. In a randomized, placebo controlled clinical trial, the non-steroidal anti-inflammatory agent diflunisal was compared to placebo, diflunisal reduced progression of neuropathy significantly in patients with hereditary ATTR amyloidosis [55]. However, there were significant toxicities associated with use of diflunisal, including renal injury, in this study. Use of diflunisal is "off-label", but is certainly used at this time for management of some patients with ATTRv amyloidosis.

More recently, two parenteral agents have been introduced for the treatment of ATTR amyloidosis with polyneuropathy, both of which work by reducing messenger RNA for TTR, and thus reducing production of the amyloidogenic TTR protein. Inotersen, an anti-sense oligonucleotide that binds up TTR mRNA, is administered subcutaneously once weekly, and was approved on the basis of the results of a pivotal randomized clinical trial. In that study, the Neuro-TTR trial, patients randomized to Inotersen demonstrated sustained reductions in transthyretin protein production, and statistically significant improvement in quality of life [56]. Similarly, Patisiran is a small, interfering RNA molecule given intravenously once every three weeks. In the Apollo study, patients with hereditary ATTR polyneuropathy were randomized to receive Patisiran versus placebo. Results documented a clinical improvement in neuropathy at eighteen months for patients treated using Patisiran versus placebo [57]. Both Inotersen and Patisiran are currently approved for treatment of ATTR polyneuropathy. In sum, for the estimated 50,000 people living with hereditary ATTR amyloidosis, there are now a number of medical treatment options with less mortality risk than liver transplant.

4. Other hereditary amyloidosis

Since 1990, several other molecules with mutations that predispose to misfolding have been discovered as rare causes of amyloidosis. These include hereditary renal amyloidosis due to mutations in lysozyme [58] giving rise to ALys Amyloidosis; mutations in fibrinogen, giving rise to AFib amyloidosis [59] apolipoproteins AI, giving rise to AApoAI amyloidosis; mutations in Apolipoprotein AII, giving rise to AApoAII amyloidosis [60]; mutations in the protein gelsolin, giving rise to AGel amyloidosis [61].

In addition, leukocyte chemotactic factor-2 related amyloidosis is an unusual amyloid disorder associated primarily with chronic kidney disease. Originally characterized in 1998, human LECT2 is a protein that is predominantly synthesized by hepatocytes. As well as having neutrophil chemotactic properties, it also appears to participate in repair of tissue injury. Since the beginning of the 21st century, an increasing number of cases of patients with localized renal amyloidosis associated with chronic kidney disease have been found, on chemical analysis, to

have amyloid comprised largely of LECT2. There is a marked over-representation of Hispanic patients with LECT2 amyloidosis, in particular patients with backgrounds from Mexico. Although the majority of these unusual cases have renal involvement only, investigators at the University of California reported a case with both renal and pulmonary involvement [62]. Genetic polymorphisms in the LECT2 gene have been identified that appear to predispose to the development of amyloidosis, and the over-representation of Hispanic patients suggests that there is a genetic component to this disease process. However, the precise etiology of the over-expression and deposition of LECT2 has not been fully established as of this writing.

5. Dialysis associated amyloidosis

Dialysis-related amyloidosis ("DRA") is a relatively common complication of chronic renal dialysis therapy, with the deposition of amyloid fibrils that are composed primarily of the molecule β2 microglobulin ("β2M"). β2M is typically hydrogen bonded to the MHC class I structure present on nucleated cell surfaces, but urea in the blood can break that bond, with β2M then circulating in the plasma. In 1975, carpel tunnel syndrome was recognized as a complication of long term hemodialysis [63]. Within a decade, this was found to be associated with histology findings of amyloid [64], and β2M was found to accumulate in patients maintained on dialysis. Soon thereafter, the amyloid deposits seen in this setting were documented to be composed in large part of β2M [65]. The amyloid in the setting of chronic renal dialysis therapy is deposited in osteoporosis-articular structures and in visceral organs, particularly at the wrists, the sternum, the knees, and the kidneys. Bone cysts also occur. Under normal physiological conditions, β2M is eliminated through glomerular filtration and subsequent reabsorption and catabolism by the proximal tubules. In general, the serum level of β2M is inversely related to the glomerular filtration rate; therefore, in end-stage renal disease patients, β2M levels may increase up to 60-fold. The β2M may then mis-fold, polymerize, and become deposited in the tissues; mis-folded intermediate forms of β2M have been reported [66], particularly truncated forms of β2M that lack the six N-terminal amino acids [67]. It is difficult to determine the true prevalence of DRA, because formal evaluation is often not undertaken. DRA is relatively common in patients maintained on long-term hemodialysis, typically for at least several years, but it has also been documented in patients undergoing continuous ambulatory Peritoneal dialysis. Aggressive dialysis, to reduce the chronic elevation of β2M is a costly, but effective, management approach.

Instances of localized deposition of amyloid – as distinct from systemic amyloidosis – were clearly recognized as a distinct clinical entity only in the twentieth century. Among the earliest reports of localized amyloid deposition is that by Gellerstedt in the year 1938 [68]. He described Congo Red positive amyloid deposits in the islets of langerhans within the pancreas, in a diabetic patient. Since that time, localized amyloidosis has been reported to occur, in rare cases, in nearly every organ in humans. However, the most commonly reported sites, by far, are the skin and the upper aero-digestive tract. Weidner and colleagues from Germany reviewed the literature regarding localized cutaneous amyloidosis, and identified small case series and case reports dating back to the year 1985 [69]. Similarly, the upper aero-digestive tract is a relatively frequent site for identification of localized amyloid deposits, in the absence of systemic disease. However, these cases are still fairly rare

overall, in general, and information regarding localized amyloidosis of the upper aero-digestive tract is primarily derived from case reports and small series [70]. It is possible that the medical literature may be skewed, and upper aero-digestive tract amyloid deposits may be over-represented, due to the fact that mass lesions in the upper aero-digestive tract give rise to symptoms even when the lesions are quite small, but there are a relatively large number of case reports of localized amyloid in the regions from the oropharynx to the pulmonary carina. In a retrospective review from Germany, the larynx was the most commonly involved site, although in that series localized disease was also seen in the tongue, trachea, and pharynx [71]. Similarly, there are many reports of localized amyloid in the lower gastrointestinal tract, likely due in part to incidental discovery during screening colonoscopy for colon cancer. An early autopsy series reported by Ravid and colleagues, published in the year 1967, detailed the findings from 391 necropsies performed at Tel HaShomer Hospital [68, op cit]. Most cases of localized amyloidosis are found to be AL amyloid, but AA amyloid may also be seen. It remains unclear why some patients with localized AL amyloid may not progress to systemic amyloid, nor why there is a tropism for the amyloid deposition to the specific sites in such cases. This is a fertile area for additional research.

6. Cerebral vascular amyloidosis

Cerebral amyloid angiopathy is a disorder in which there is accumulation of amyloid within the walls capillaries, as well as of small and medium sized arterial vessels within the nervous system. This results in weakening of the vessels over time, and the disorder typically presents in the elderly. Clinically, it may manifest as either micro-hemorrhages, which can result in cognitive impairment and possibly vascular dementia, or may manifest as intra-cerebral hemorrhage, which may be catastrophic. An early description of cerebral amyloid angiopathy was published by Ishiguro and colleagues in the year 1984. In this autopsy series, the authors identified seven cases in which patients presented with either intra-cerebral hemorrhage or with dementia. At autopsy, amyloid deposition was observed in the smaller arterial vessels, including at the sites of hemorrhage [72]. Later analysis of the amyloid showed this to be deposition of Amyloid Precursor Protein (APP), a membrane protein that is present in high concentrations at neuronal synapses. Proteolysis of APP results in amyloid Aβ polypeptides. These are a principle component of the amyloid found in cerebral vascular amyloidosis; amyloid Aβ polypeptide is also a component of the amyloid deposits in Alzheimer's Disease. However, in Alzheimer's Disease, the Aβ fragments typically extend to amino acids position 42, whereas in cerebral vascular amyloidosis, the Aβ fragments extend only to amino acid positions 39 or 40 [73]. Neurofibrillary tangles, a hallmark of Alzheimer's Disease, are not seen in cerebral vascular amyloidosis.

7. Amyloid in Alzheimer's disease

Amyloid deposition is an integral aspect of the histopathology of Alzheimer's Disease. However, the amyloid is only one aspect of the dramatic microscopic changes seen in the brain when affected by Alzheimer's disease. Alzheimer's disease is discussed in detail in other chapters of this volume, and therefore will not be reviewed here. A timeline of the many of the most significant observations and developments in the amyloidoses is provided in **Figure 1**.

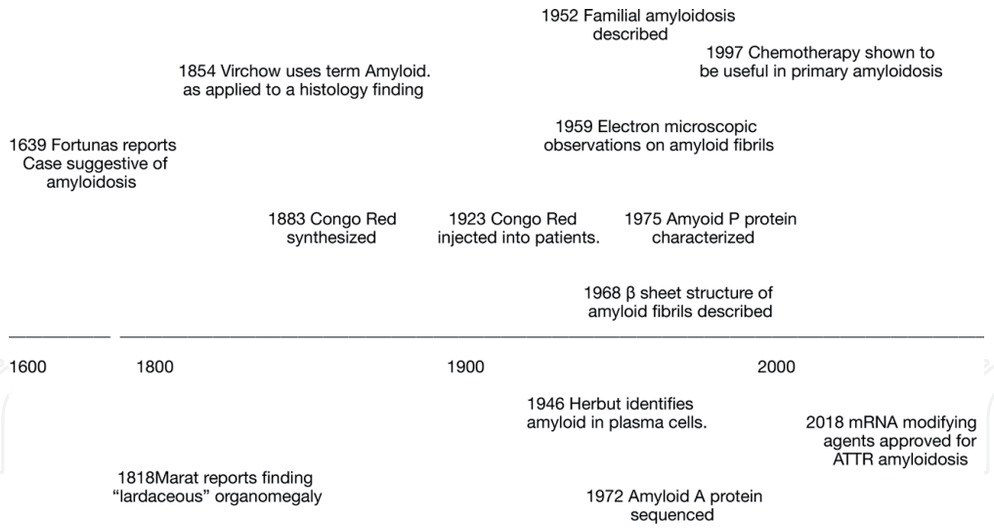

Figure 1.
A timeline of major developments in Amyloidosis.

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