

AMYLOIDOSIS AWARENESS

For patients and their support network, including physicians, nurses and medical students



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While the information herein is meant to be accurate, the medical sciences are ever advancing. As such, the content of this publication is presented for educational purposes only. It is not intended as medical advice. All decisions regarding medical care should be discussed with a qualified, practicing physician.

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Cover image: Amyloidosis often occurs in middle-age and older individuals, but also in patients in their 30s or 40s, and occasionally even younger.

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1. ONE MINUTE OVERVIEW

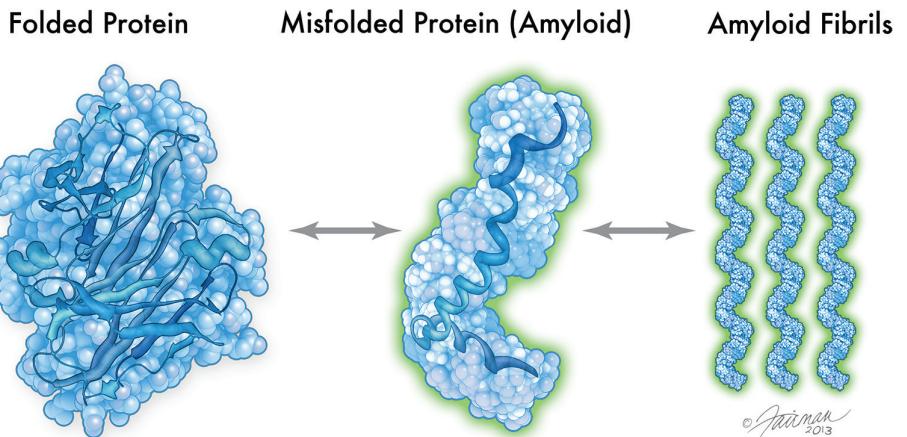
All the normal proteins in our body are biodegradable and recyclable. Amyloidosis is a disease in which abnormal proteins misfold and form fibrils that are resistant to being broken down. The amyloid protein fibrils deposit and accumulate in the body's tissues. If amyloid builds up in the kidney, heart, liver, gastrointestinal tract, or nerves, it causes those organs to function poorly. The symptoms of amyloidosis are the result of the abnormal functioning of the organs involved. Typically, patients will have some of the following symptoms: unexplained weight loss, fatigue, shortness of breath, foamy urine, heart palpitations, swelling (in the abdomen, ankles and legs), easy bruising, carpal tunnel syndrome, lumbar spinal stenosis, or numbness and tingling in the hands and feet. These are manifestations of damage to, or dysfunction of, the underlying organs from the amyloid protein. Treatments are designed either to stop the production of the amyloid proteins, or to stabilize them to prevent misfolding and fibril formation. There is also ongoing research to develop therapies with the potential to dissolve existing amyloid deposits. Left untreated, the systemic disease can be life-threatening. Therefore, early, and accurate diagnosis is the key to promoting good outcomes.

2. WHAT IS AMYLOIDOSIS?

Throughout our lifetime, our DNA codes for the production of small molecules called proteins. These proteins provide the structure and function for nearly all of life's biological processes. Enzymes that permit our cells to function, hormones that affect our body's growth and metabolism, and antibodies that form our immune response are all examples of proteins in action. Just about everything in our bodies – from the color of our eyes, to carrying oxygen in our blood, to whether we can digest certain foods – is determined by the proteins we make.

Once produced within the body, proteins will naturally fold into a particular shape. This natural folding of a protein is what allows it to function normally. Put simply, when proteins are folded properly, they work as intended, and we enjoy relatively good health. When proteins are misfolded, it affects our body's ability to function, and problems may arise over time.

Misfolded proteins can be produced because of changes in our genetic code (mutations), factors related to chronic inflammation, or simply as a result of aging. Regardless, our bodies are usually capable of identifying and removing these abnormal proteins. In some cases, though, we either produce more of the abnormal proteins than our body can handle, or we are not able to break down and clean up the proteins at all. Errors in protein production and processing (called proteostasis) are associated with many diseases.



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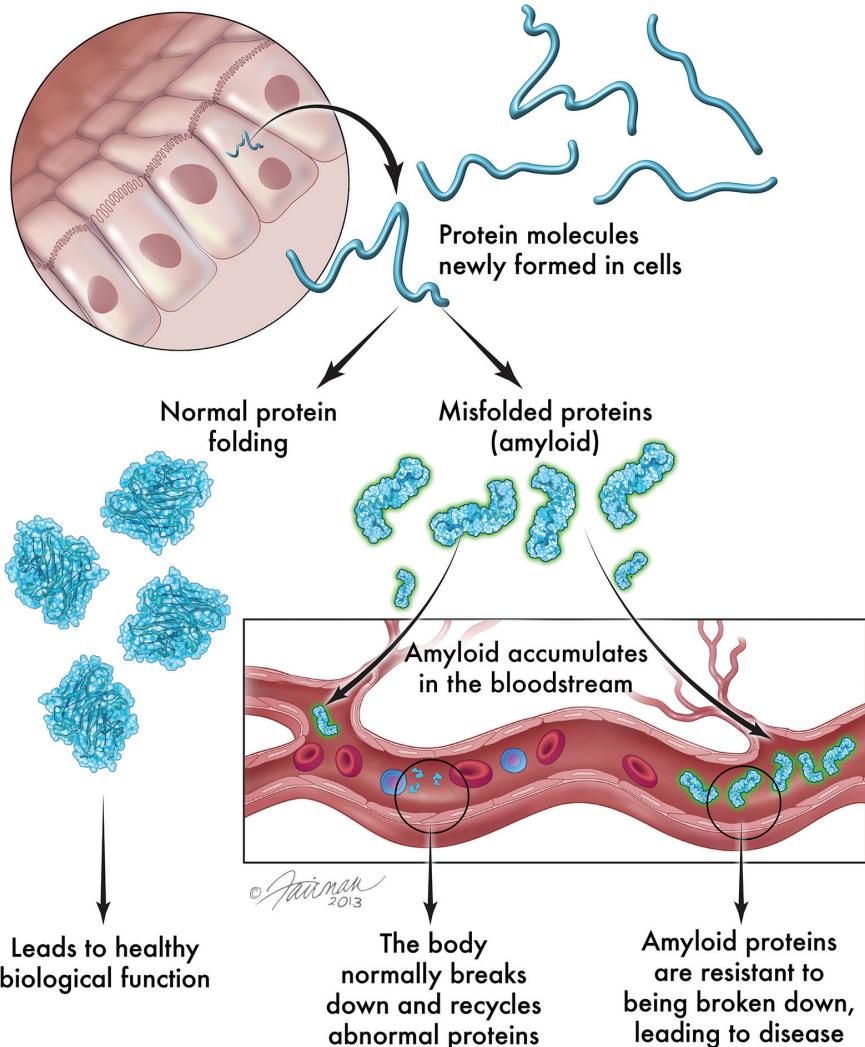
Scanning electron
microscopic view
of amyloid fibrils

Amyloid is a substance caused by the misfolding of proteins. Amyloid binds together into rigid, linear fibers (fibrils) that deposit in the tissues and organs.

Broadly speaking, amyloidosis (pronounced ‘am-uh-loy-doh-sis’) is one class in a growing list of protein misfolding disorders. While there are many distinct types of amyloidosis, in all cases the misfolded proteins, called amyloid, take on a particular shape that makes it difficult for the body to break down. The name “amyloid” derives from the Latin “starch-like” because it used to be thought that it was comprised of complex sugar molecules. It was later shown to be made of misfolded proteins. The misfolding allows the amyloid proteins to bind together to form rigid, linear fibers (or fibrils) that accumulate in our body’s organs and tissues. Depending on where the amyloid builds up, such as in the kidney, heart and nerves, different symptoms and potentially life-threatening conditions occur.

While amyloidosis has been known since the 19th century, it is only within the last few decades that our understanding of it has matured. Presently, more than 35 different proteins, when misfolded, have been identified to be associated with amyloidosis. The major forms are described in the next section. Additional types of precursor proteins that can lead to amyloid formation continue to be discovered through ongoing research.

Although historically believed to be a rare condition, recent research suggest certain types are more common than previously thought. For instance, the incidence of AL amyloidosis is about 50,000 per year worldwide, with 3,000 people diagnosed in North America alone. This is about 1/5 of the incidence of multiple myeloma, and of similar incidence to that of Hodgkin’s disease or chronic myelogenous leukemia. ATTR amyloidosis, which has



Misfolded proteins can be produced because of genetic causes, or because of other factors related to chronic inflammation or increasing age.

hereditary and age-related forms, is more common than AL, accounting for as much as 10-15% of cases of congestive heart failure in elderly people. Still, the diagnosis is often overlooked. Because it is perceived to be a very rare disease, medical students and physicians may not expect to see amyloidosis in their practice. Moreover, because the nonspecific symptoms (e.g., being tired or out of breath) may be mistakenly attributed to more common causes of lung and cardiovascular disease, it is very likely that the actual prevalence of amyloidosis is greater than now recognized. It is imperative for clinicians and pathologists to consider amyloidosis in patients with suggestive symptoms (discussed in section 4). Given the unique staining and chemical properties of amyloid proteins, it is a simple matter to test for the disease. Early, accurate diagnosis is essential for patients to benefit from available treatments (discussed in section 5).

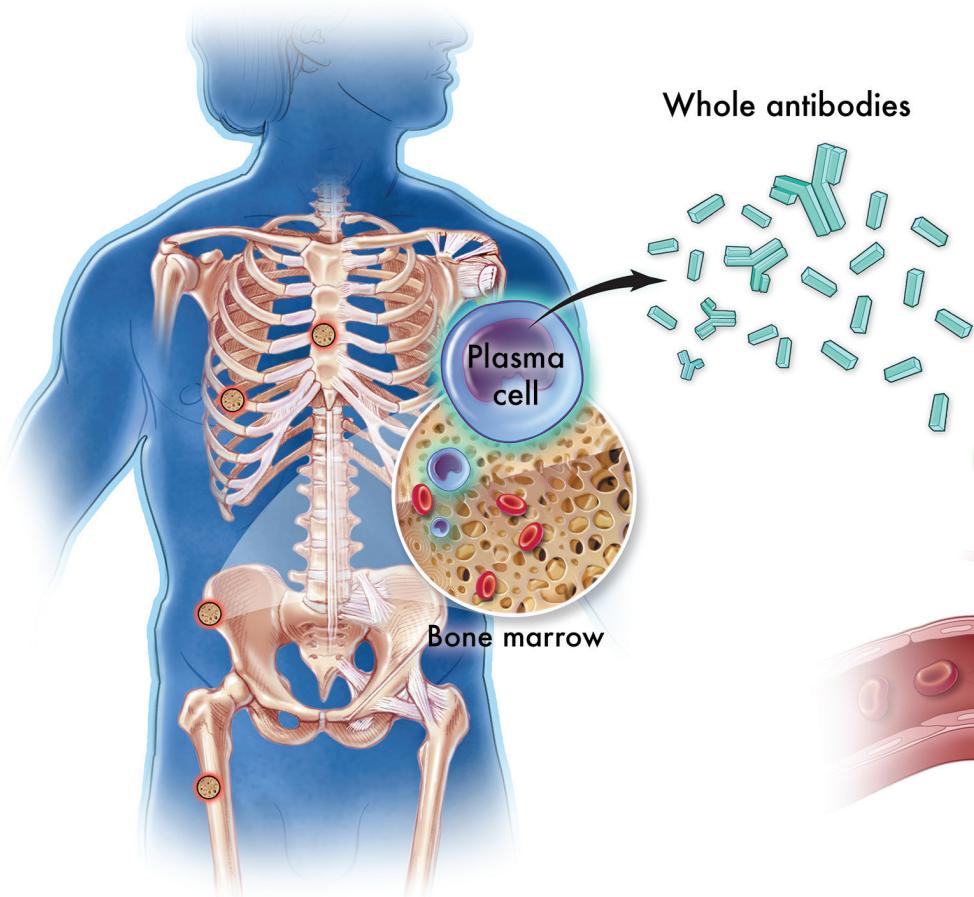
3. TYPES OF AMYLOIDOSIS

There are many different proteins in our bodies that can become misfolded to produce amyloidosis. The tendency to form abnormal proteins can be inherited from our parents, or even arise from spontaneous DNA mutations. In some instances, amyloidosis results from chronic inflammatory or infectious diseases, or (rarely) long-term kidney dialysis. A significant portion of cases are caused by a bone marrow condition that has similarities with a type of blood cancer called multiple myeloma.

As the amyloid proteins accumulate in our bloodstream, they ultimately deposit in organs and tissues as amyloid fibrils. These deposits may impair multiple organ systems or be limited (localized) to one area of the body. Amyloid commonly deposits in the kidney, heart, and nerves. The liver, spleen, gastrointestinal tract, skin, and airway can also be affected. Although the precursor proteins that lead to amyloidosis come in various shapes and sizes, they all share a similar misfolded structure when they form amyloid deposits. The different types of amyloidosis can be classified according to the precursor protein involved. As seen in Table 1 (opposite page), a convenient naming system is used, such that the prefix “A” refers to amyloid, followed by an abbreviation for the causative protein. For example, AL designates amyloid derived from light-chain antibodies; AA designates serum amyloid A protein; and ATTR designates amyloid from transthyretin.

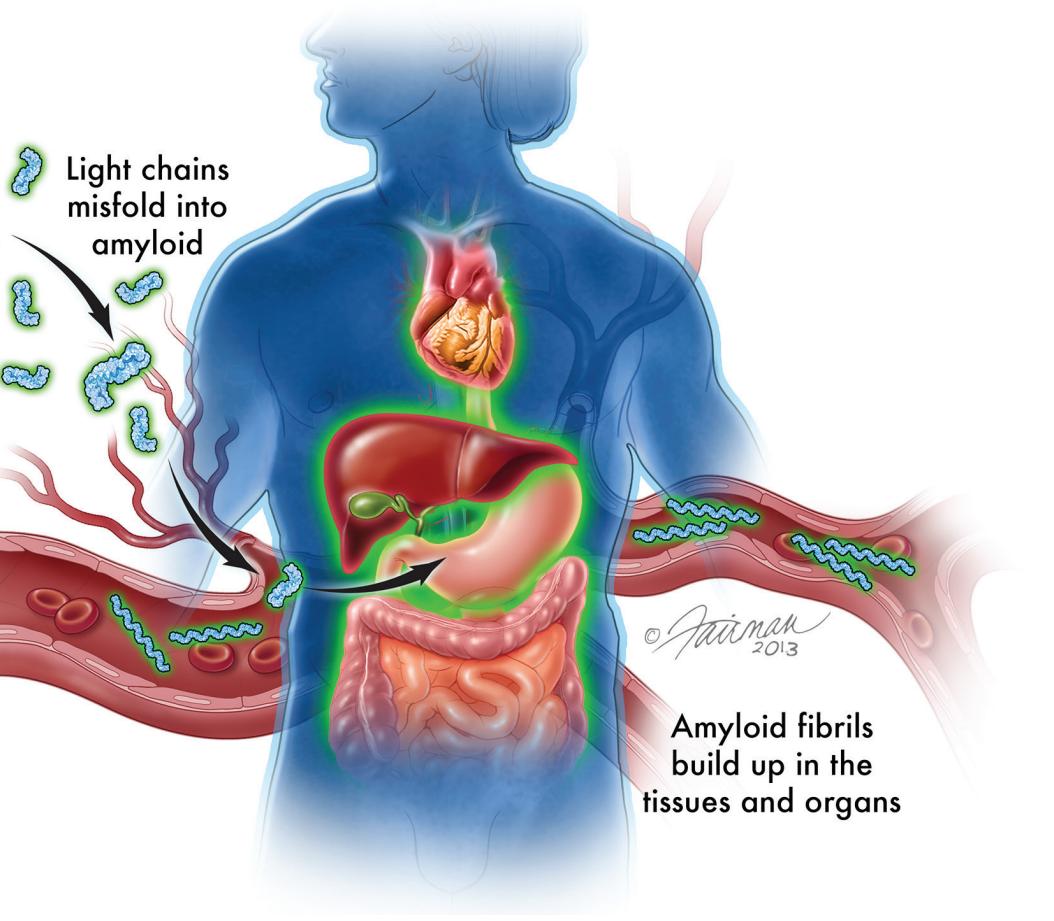
TYPE	SOURCE OF AMYLOID (Precursor Protein)	SYNDROME
AL, AH, ALH	Plasma cells in the bone marrow (Immunoglobulin light or heavy chains, or both)	Primary form of amyloidosis, similar to multiple myeloma, affecting the kidneys, heart, liver, gastrointestinal tract, and nerves.
AA	Circulating inflammatory protein (Serum amyloid A)	Secondary to chronic inflammatory and infectious diseases, affecting the kidneys and liver.
ALECT2	White blood cells (Leukocyte chemotactic factor 2)	Particularly common in some ethnic groups, affecting the kidneys and liver.
A β 2M	Circulating serum protein (β_2 -microglobulin)	Dialysis-related, affecting the joints and tendons.
ATTR	Mutant and wild-type protein produced in the liver (Transthyretin)	Hereditary with over 130 mutations, affecting the nervous system, heart, and kidneys. The Val122Ile mutation is common in Black Americans, causing cardiac disease. A non- hereditary form (called wild-type) causes cardiac disease in the elderly.
AFib	Mutant protein produced in the liver (Fibrinogen A α -chain)	Hereditary, affecting the kidneys.
AApoAI	Circulating serum protein (Apolipoprotein AI)	Hereditary, affecting the liver, heart, kidneys, and nerves.
ALys	Circulating serum protein (Lysozyme)	Hereditary, affecting the gastrointestinal tract and kidneys.
AGel	Circulating serum protein (Gelsolin)	Hereditary, affecting the eyes, skin, nerves, and kidneys.
Localized	Plasma cells in local tissues (Immunoglobulin light chains)	Mostly occurs in the bladder, skin, and airways.

Table 1: Examples of amyloidosis. The naming system combines an “A” for amyloid with an abbreviation for the protein underlying the condition.



In AL amyloidosis, plasma cells in the bone marrow produce too many “free light chain” antibodies. These proteins misfold into amyloid, accumulate in the blood, and deposit in many organ systems.

Below is a brief description of AL amyloidosis, AA amyloidosis, various forms of hereditary amyloidosis, wild-type ATTR amyloidosis; as well as ALECT2, dialysis-related, and localized amyloidosis.



AL Amyloidosis

AL (or primary) amyloidosis was historically thought to be the most common form of the disease; however, ATTR Wild-Type has recently become the type diagnosed most often, espe-

cially in older adult patients. AL begins in the bone marrow, the soft tissue that fills the cavities of our bones where red and white blood cells are formed. One kind of white blood cell, called plasma cells, produces antibodies that protect us from infections. These antibody proteins (immunoglobulins) are made up of light and heavy chain molecules. Normally, our plasma cells produce whole antibodies, and our body breaks down these proteins and recycles them after a short time. In AL, though, too many unassembled, misfolded light chains are being made. These “free light chains” (and, in rare cases, misfolded heavy chains) cannot be broken down efficiently. They bind together to form amyloid fibrils that build up in the extracellular space of organs and tissues. In this way, the body’s normal functioning is impaired. Problems typically arise in one or more organs: kidney, heart, liver, spleen, nerves, intestines, skin, tongue, soft tissue, or blood vessels.

AA Amyloidosis

AA (or secondary) amyloidosis results from increased levels of the circulating serum amyloid A protein. Serum amyloid A elevates in our blood as a natural response to infection and inflammation. In general, if a patient has an infection or inflammatory condition for six months or more, he or she is at risk for developing AA. Examples of chronic inflammatory conditions which can give rise to AA amyloidosis include rheumatic disease (such as juvenile and adult rheumatoid arthritis), inflammatory bowel disease, tuberculosis, chronic osteomyelitis, lupus, and hereditary fever syndromes such as familial Mediterranean fever (FMF). With treatment of the underlying inflammatory condition, this form of amyloidosis is less commonly

encountered. Amyloid deposition usually begins in the kidneys, but the liver, spleen, lymph nodes, heart and intestines can also be affected.

Hereditary Transthyretin Amyloidosis (ATTRv or hATTR)

Heredity amyloidosis, as the name implies, results from a change (mutation or variant) in the gene coding for the affected protein. Mutations are usually inherited from one's parents.

Rarely, a brand-new mutation which was not inherited from a parent occurs in a person's DNA which can then be passed down to future generations. The most common form of hereditary amyloidosis is due to mutations in the gene coding for transthyretin (TTR) protein produced mainly in the liver. This type is often referred to as ATTRv or "hereditary ATTR" but has also been written as hATTR or ATTRm. TTR is a protein that helps to transport thyroxine (a thyroid hormone) and retinol (vitamin A) around the body. There are more than 130 known mutations of TTR that cause the protein to become unstable and misfold into amyloid. Various organs are affected, especially the nervous system and heart, with symptoms most often occurring in mid- to late life. If the nerves are primarily affected, the condition is known as hereditary or variant amyloid with polyneuropathy (ATTRv-PN); if the heart is primarily affected, it is known as hereditary or variant amyloid with cardiomyopathy (ATTRv-CM).

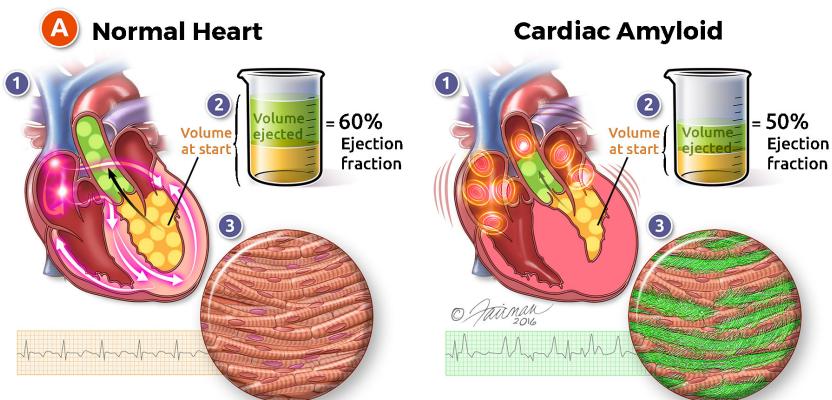
The most studied mutation of TTR worldwide is called Val30Met, which causes damage to the nerves and sometimes problems with the heart. Another common mutation in the

United States is Thr60Ala which causes thickening of the heart muscle and nerve injury and commonly occurs in individuals of Irish descent. While transthyretin-mediated amyloidosis (ATTRv) occurs in families of nearly every ethnicity, there is one TTR variant, Val122Ile, that is particularly common in people of African American and Afro-Caribbean populations. It is estimated that 3-4% of Black Americans carry this variant of the TTR gene, which equates to over 1.35 million individuals who carry the variant in the US alone. Because of this, ATTRv makes up a large proportion of the total cases of amyloidosis occurring in Black Americans (25% of all cases of amyloidosis in this population). It is important for physicians to consider this as a possible cause of congestive heart failure in Black Americans.

Wild Type Transthyretin Amyloidosis (ATTRwt)

Wild Type transthyretin amyloidosis (ATTRwt- formerly called senile systemic or age-related) is most often a late onset disease that is acquired, not inherited. In other words, there is no mutation in the TTR gene in this form of ATTR. Amyloid deposits accumulate in the body from normal (wild type) transthyretin proteins. ATTRwt amyloid deposits build up mostly in the hearts of older adults. Affected patients usually have slowly progressive cardiac symptoms. Whether hereditary or wild-type, TTR-mediated amyloidosis is thought to be more common than AL amyloidosis, though it often goes undiagnosed. For example, cardiac ATTR deposits are found in up to 20-25% of all people over age 75 years. The buildup is significant

enough in some cases to cause congestive heart failure, even though the pump function of the heart may appear preserved on diagnostic studies like an echocardiogram (an ultrasound of the heart). Despite the prior usage of the word “senile” to describe this group of amyloidosis subtypes, this condition has no relationship to senility or dementia.



ATTR often causes heart failure with preserved ejection fraction (HFpEF).

ALECT2 Amyloidosis

One of the most recent additions to the list of amyloid proteins is ALECT2, derived from a protein made by white blood cells (leukocytes). This disease is most often seen in the Native American, Mexican and First Nation people. It has also been seen in both India and the Middle East. Although there is ongoing research about why LECT2 misfolds and forms amyloid deposits in affected people, there is no definite evidence that ALECT2 is the result of genetic mutations. As with the age-associated types of systemic amyloidosis, ATTRwt and those

listed in the section below (“Other Types of Amyloidosis”), amyloid deposits are formed from non-mutated (wild-type) proteins. This form of the disease may be mis- or underdiagnosed. One study, in fact, suggested that ALECT2 is a fairly common type of kidney amyloid, especially among patients of Mexican heritage. In an analysis of amyloid-containing kidney specimens over the last 8 years, ALECT2 was the third most common type found (2.5%), as compared to AL (86%), AA (7%) and ATTR (1.4%).

A β_2 M Amyloidosis

A β_2 M (or dialysis-related) amyloidosis can occur in patients suffering from kidney failure, who have been on dialysis for many years. A circulating serum protein, beta-2 microglobulin (β_2 M), accumulates in the blood because it is unable to pass through the dialysis filter. This may be less of an issue with modern dialysis filters, and the incidence of this form of amyloidosis appears to be declining as a result. In this form of amyloidosis, fibrils made up of β_2 M builds up in tissues, particularly in the joints and tendons. This causes pain, stiffness, and fluid in the joints, as well as carpal tunnel syndrome.

Other Types of Amyloidosis

In addition to the many different hereditary TTR mutations seen in ATTRv, there are other gene mutations for different proteins that lead to amyloidosis. Though very rare, some of these include: AFib (from fibrinogen A α -chain; not to be confused with the common heart problem atrial fibrillation, which is also commonly referred to as “A-Fib”); AApoAI (from apolipoprotein

AI); ALys (from lysozyme); and AGel (from gelsolin).

Also, while ATTRwt is the most common age-related form of amyloidosis, other examples of age-related amyloidosis include: APro (from prolactin); ACal (from calcitonin); AIAPP (from amylin); and AANF (from atrial natriuretic factor). In each of these, the amyloidosis is caused by the misfolding of non-mutated wild type proteins (just like in ATTRwt).

Localized Amyloidosis

Though the major forms of systemic amyloidosis are described above, it is important to recognize that amyloid deposits may occasionally occur in isolated areas of the body, without evidence of a systemic disease. These localized, tumor-like deposits most often occur in the bladder and airways (e.g., trachea or lungs). Deposits have also been diagnosed in and around the eye, gastrointestinal tract, skin, and breast. Most commonly, the localized amyloid deposits are made up of immunoglobulin light chain proteins (like in AL amyloidosis). However, in localized amyloidosis, the abnormal plasma cells producing the amyloid light chains are in the affected tissues, not in the bone marrow.

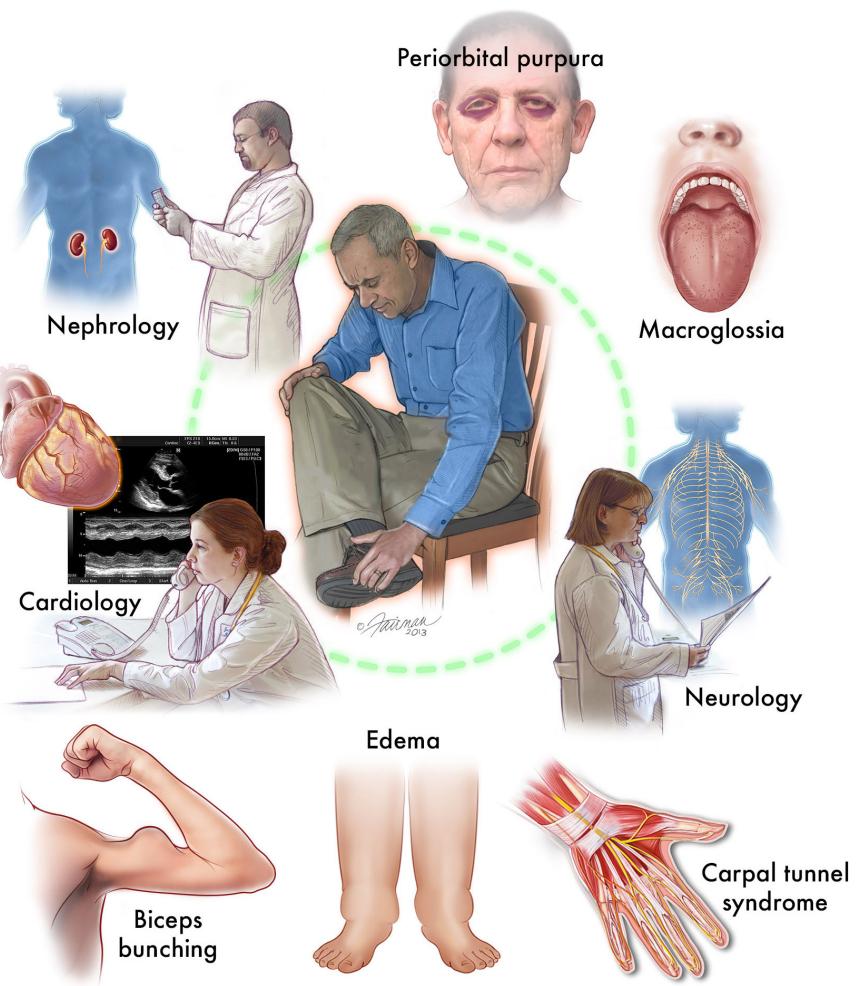
Other non-systemic types of amyloidosis are associated with hormone proteins, aging, or specific areas of the body. One special case of localized amyloidosis is cerebral amyloid angiopathy (CAA). While the cause is still unknown, in some individuals, CAA may be hereditary. Amyloid protein deposits in the walls of the brain's arteries, increasing the risk of stroke, dementia, and bleeding. Although this neurological condition is mostly seen in older patients, it is unrelated to Alzheimer's disease.

4. DIAGNOSIS

In some respects, amyloidosis is often difficult to recognize. Its symptoms are vague and nonspecific, often mimicking those of more common conditions. For instance, shortness of breath can be an indicator of heart disease associated with much more common medical problems such as hypertension, heart failure or lung disease. Another example is protein in the urine (“proteinuria”), which can occur in amyloid-related kidney injury. Because much more common diseases like diabetes can also cause protein in the urine, health care providers do not normally think of amyloidosis first.

Amyloidosis typically appears in middle-age and older individuals, but it can also occur during one's 30s or 40s, and occasionally even younger. Depending on where in the body they occur, amyloid deposits can cause weight loss, fatigue, shortness of breath, dizziness upon standing, swelling in the ankles and legs, numbness and tingling in the hands and feet, foamy urine, alternating bouts of constipation and diarrhea, and feeling full quickly after eating. Carpal tunnel syndrome can often be seen with AL and ATTR amyloidosis patients, and lumbar spinal stenosis can be frequent with ATTR patients as well. Also, if a patient bruises easily, especially around the eyes (periorbital purpura), or has an enlarged tongue (macroglossia), AL amyloidosis is very likely the underlying cause.

Even as a tell-tale group of symptoms persists and worsens, many physicians do not consider (or remember) an



Symptoms are often vague, mimicking those of other common conditions. Therefore, a multidisciplinary approach among physician specialists is essential for diagnosis. In some cases, telltale signs of amyloidosis are an enlarged tongue (macroglossia) or bruising around the eyes (periorbital purpura).

uncommon, insidious disease such as amyloidosis. It is not unusual for an affected individual to see several physicians over a period of many months or even years before a biopsy (tissue sample) is taken. Some patients develop organ failure before a proper diagnosis is made. When a biopsy is performed, it is extremely important that the pathologist is informed of the suspected diagnosis to ensure appropriate testing of the sample (see next section). Without the clinical information, the pathologist may only consider more common diagnoses and miss amyloidosis.

While amyloidosis can affect just a single organ, it often causes systemic problems (i.e., it affects more than one organ system). The organs most often involved in AL amyloidosis are the kidneys (about 70% of patients), heart (60%), nervous system (30%), and gastrointestinal tract (10%). Therefore, the combination of kidney, heart, nerve, gastrointestinal and/or liver disease – with no other obvious cause – should prompt physicians to test for amyloidosis.

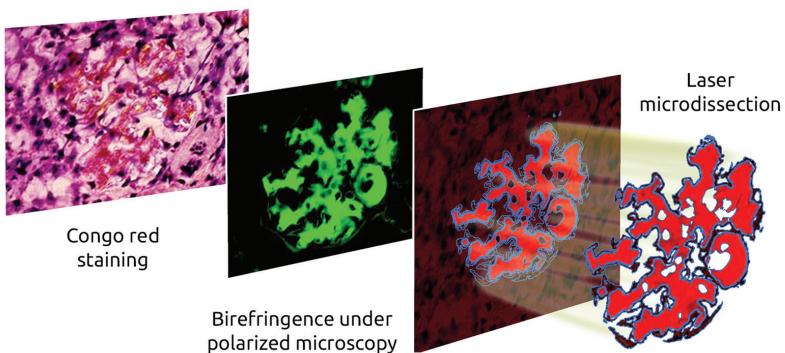
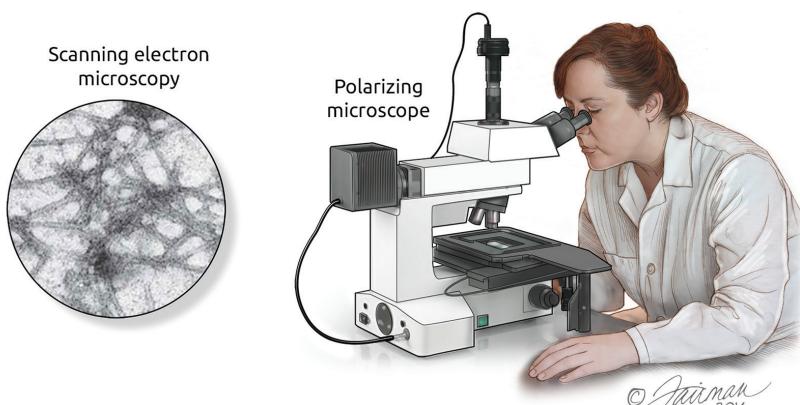
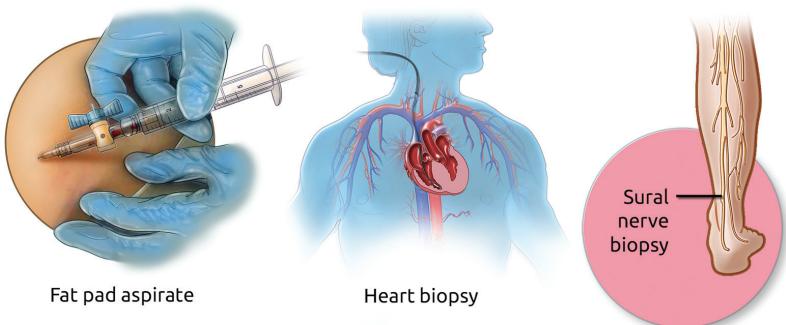
The four most common clinical settings in which amyloidosis should be considered are:

1. *Loss of massive amounts of protein in the urine (proteinuria; also called nephrotic syndrome) This suggests kidney involvement.*
2. *Stiff or thickened heart (restrictive cardiomyopathy) as seen on echocardiogram; low voltage seen on electrocardiogram (EKG or ECG); irregular heartbeat (arrhythmia) that is resistant to conventional treatment, often associated with normal or low blood pressure; or unexplained heart failure. These findings suggest heart involvement.*

3. *Enlarged liver (hepatomegaly) without alcohol consumption or other explanation, often with abnormal liver blood tests, including elevated alkaline phosphatase. This suggests liver involvement.*
4. *Numbness, tingling, or pain in the fingers or toes (peripheral neuropathy), carpal tunnel syndrome (especially when affecting both hands), or alternating bouts of constipation and diarrhea with or without feeling light-headed due to a drop in the blood pressure when standing up (autonomic neuropathy). These symptoms could suggest nerve involvement.*

Testing for Amyloidosis

Once amyloidosis is suspected, it can sometimes be identified, if present, with a very simple office procedure. Early detection and accurate evaluation are essential for patients to benefit from the many therapies now available (discussed in the next section). Blood and urine tests may provide hints about the diagnosis, but the gold standard for detecting amyloid deposits is to perform Congo red staining on a tissue sample. While biopsies can be taken from the gums, nerves, kidney, liver, tongue, heart, rectum, or other organs, the easiest way to get a tissue sample is to aspirate fat from the abdomen. In this minimally invasive procedure, the skin of the belly is numbed with a local anesthetic, and a needle is used to perform a mini liposuction of fat from under the skin. The sample obtained is usually about the size of a chickpea or a pencil eraser. Because of the common misfolded structure of all amyloid, it has a pink color when dyed with Congo red stain and viewed under a standard microscope, and a characteristic apple-green appearance when viewed with a polarizing microscope.



The gold standard for detecting amyloidosis uses Congo red stain on a tissue sample, which appears apple-green when viewed with a polarizing microscope. Laser microdissection followed by mass spectrometry can determine the type of amyloid in virtually 100% of cases.

This signature technique can diagnose amyloidosis in 70-80% of patients with AL amyloidosis, but only about 15-20% of patients with ATTR amyloidosis. A negative fat aspirate does not rule out amyloidosis.

In the case of AL amyloidosis, a fat pad aspirate and bone marrow biopsy are two of the initial tests performed. If the fat pad aspirate and bone marrow biopsy are negative for amyloidosis, but suspicion of the disease remains high, a direct biopsy of the involved organ (e.g., the heart, kidney, or liver) should be done. If amyloid is present in the biopsied tissue, Congo red staining will yield a definitive diagnosis in nearly 100% of cases. It is important that the pathologist working on the biopsy has experience with Congo red staining, as over-staining the tissue sample may give false results. Visualizing the tissue with an electron microscope will show the classic structure of amyloid fibrils and is helpful in confirming its presence.

Typing the Amyloidosis

Proving that amyloid is present in an organ is only the beginning of the process. Next it must be determined what kind of amyloid is causing the disease to plan for an appropriate, individualized treatment. In all cases, identifying the amyloid type must be based on evaluation of the abnormal protein deposits in the affected tissues. It is recommended that this additional testing on the tissue sample be performed at a specialized amyloidosis center (see Section 6) with sophisticated diagnostic tools at their disposal. Consultation with experts at such a center should also be considered if,

after extensive local testing, a diagnosis of amyloidosis remains suspected but not established.

A simple blood test to measure the levels of kappa and lambda serum free light chains when coupled with measures for monoclonal proteins in the serum and urine (serum and urine electrophoresis with immunofixation) will show disproportionately elevated levels of one type or the other in roughly 98% of patients with AL amyloidosis. This test is often one of the earliest ones done. A bone marrow biopsy, as mentioned above, often reveals amyloid deposits and in almost all cases, the abnormal (clonal) plasma cells which produce the defective, amyloid-forming light chains. These cells are identified using special staining (immunohistochemistry) or cell sorting techniques (flow cytometry).

If these tests are negative, other forms of the disease should be investigated including the ATTR types. Molecular and genetic testing can be performed on blood samples to see if the patient has any of the hereditary types of amyloid (e.g., TTR, fibrinogen, lysozyme, apolipoproteins AI and AII, and gelsolin). If one has such a mutation, then his or her children each have a 50% chance of inheriting it. It should be emphasized that the presence of a genetic mutation does not guarantee a person will develop amyloidosis. Also, it is possible, albeit rare, to develop non-inherited types of amyloidosis (like AL or AA) even when you are a carrier of a mutation or genetic variant associated with inherited amyloidosis, or even to develop two types of amyloidosis at the same time. The complicated detective work needed to sort out these types of cases often requires the clinical expertise and advanced diagnostic testing

only available at major amyloidosis centers.

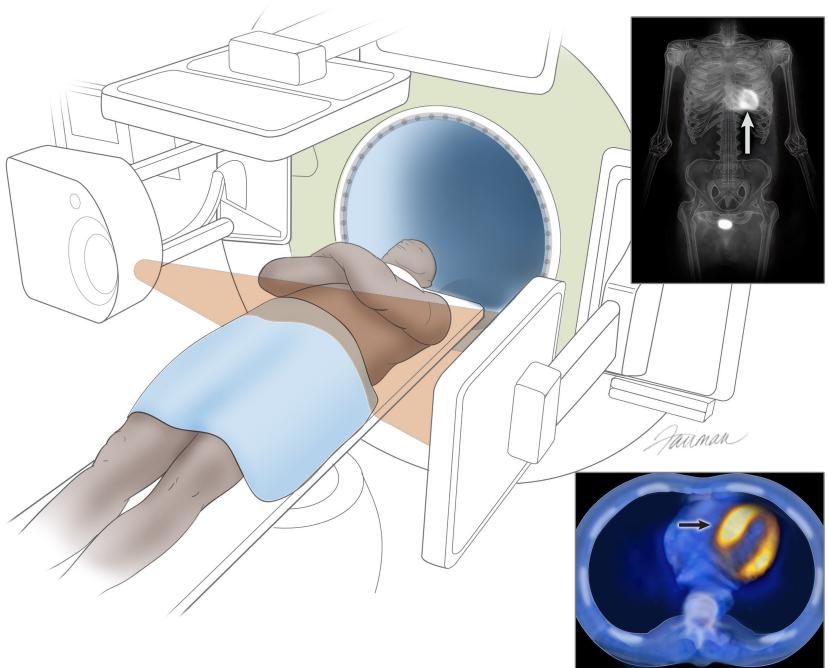
Understandably some people are reluctant to be tested for a genetic disease. In the United States, the Genetic Information Nondiscrimination Act (GINA), legislates that patients who have a hereditary predisposition for diseases, such as amyloidosis, cannot be discriminated against with respect to employment or health insurance. Patients are encouraged to meet with a licensed genetic counselor before testing.

Importantly, sometimes a patient's other medical history provides clues about the most likely type of amyloidosis. For patients with chronic inflammatory or infectious conditions, or long-term kidney dialysis, primary consideration would be AA or A β 2M amyloidosis, respectively. Recurrent strokes or progressive dementia with evidence of recurrent small brain bleeds on an MRI suggest cerebral amyloid angiopathy (CAA). In a person over the age of 50, presenting with congestive heart failure with an increased wall thickness on echocardiogram in the absence of hypertension, a primary consideration would be wild type ATTR amyloidosis (ATTRwt).

Meanwhile, recent advances in the field of proteomics promise to revolutionize the precise diagnosis of amyloidosis. Proteomics involves the study of the entire collection of proteins in an organism or environment. Unlike standard immunochemistry techniques, which in many cases fail to accurately diagnose which precursor protein is responsible for the amyloid deposits, proteomics can identify the specific protein in the amyloid deposits with or without genetic mutations. Laser microdissection followed by mass spectrometry (LDM-MS) is

the premier technique in typing amyloidosis. To perform the test, Congo red positive samples are dissected and broken down into smaller components of protein molecules (called peptides). The peptides are then analyzed using a process known as “liquid chromatography electrospray tandem mass spectrometry,” also termed mass spectrometry or LMD-MS, for short. Studies have shown that LMD-MS has the capability to identify most known amyloid proteins with virtually 100% accuracy, as well as the ability to characterize new ones. Certain forms of amyloidosis which have historically been underdiagnosed, such as the Val122Ile, the TTR variant that causes cardiac amyloidosis in Black Americans, and the wild type ALECT2 protein that causes kidney disease in patients of Mexican heritage, are readily identified using LMD-MS.

There is one circumstance in which cardiac amyloidosis may be diagnosed without a biopsy. If ATTR cardiac amyloidosis is suspected, a diagnosis can be made using a nuclear medicine scan, Tc-99m PYP (pyrophosphate scan) in the United States, DPD/HMDP in Europe. If there is abnormal tracer uptake in the heart along with typical echocardiographic or cardiac MRI findings, and if screening blood and urine tests for AL are negative, the diagnosis of ATTR can be confirmed. It should be noted that other types of amyloidosis such as AL or apolipoprotein can occasionally result in a positive nuclear medicine scan, as can other technical issues, such as blood pool. The diagnosis of amyloidosis based on cardiac imaging alone requires considerable expertise and should involve cardiologists and/or nuclear medicine specialists with experience evaluating amyloidosis.



After ruling out AL amyloidosis with serum free light chain test and serum/urine protein electrophoresis and immunofixation, a technetium-99 m-pyrophosphate bone scintigraphy scan (PYP scan) can be used as a noninvasive tool to diagnose ATTR amyloidosis.

In summary, diagnosis of a specific type of amyloidosis requires the evaluation of clinical factors such as age, ethnicity, family history, personal medical history, together with sophisticated diagnostic testing.

5. TREATMENTS

Some physicians used to assume that nothing could be done for a patient with amyloidosis. This is simply not true, especially as effective treatments have been approved by regulatory agencies in many countries.

Working with a team of physicians – including hematologists, cardiologists, nephrologists, and neurologists, among others – it is important to get a conclusive, accurate diagnosis of the disease as soon as possible. Selection of optimal therapy depends on knowing the type of amyloidosis and the organs affected, and must also take into account factors like the patient's condition, age, and personal preferences. If not treated in a timely manner, amyloid deposits will continue to damage tissues until organ failure and possibly death occurs.

Treatment of amyloidosis should be thought of as a two-part process:

- (1) *Manage symptoms to promote the patient's well-being, improve quality of life and function, and prolong survival.*
- (2) *Reduce or stabilize the amyloid protein to prevent continued formation of amyloid deposits.*

In many cases, if the production of abnormal protein is removed, existing amyloid deposits can be reabsorbed, and slowly over time organ function can be restored.

There are three general approaches to disrupt the formation and deposition of amyloid protein, which vary according to the type of amyloidosis. The most common strategy is to shut down the production of the precursor protein leading to the disease. A second

method uses drug therapy to stabilize the normal structure of the precursor protein, thus preventing it from misfolding into amyloid. A third strategy, in clinical trials as of this writing, is to target amyloid deposits directly, either through investigational immune strategies or by destabilizing the amyloid fibrils so they are more easily dissolved by the body. All therapies have side effects and amyloidosis experts should be consulted to recommend the best options.

AL Amyloidosis

For AL (or primary) amyloidosis, a frequently diagnosed form of the disease, extensive organ involvement is common. Without treatment, the average survival time is about 12-18 months, and only about 6 months for patients with severely impaired heart function.

Chemotherapy, either orally, subcutaneously, or intravenously, forms the cornerstone of treatment for AL amyloidosis. The goal is to reduce the number of plasma cells, which in turn reduces the levels of the abnormal light-chain antibody proteins those cells produce. For several years, the chemotherapy drugs melphalan (also known as Alkeran) or cyclophosphamide (Cytoxan) have been the first choice used, usually in combination with dexamethasone, a steroid which synergizes with them to kill the plasma cells. Other drugs that are used in treating multiple myeloma (a plasma cell cancer), such as bortezomib (Velcade) lenalidomide (Revlimid), pomalidomide (Pomalyst) or ixazomib (Ninlaro) also have proved effective. These therapies are also often used in combination with melphalan, cyclophosphamide, and/or dexamethasone. Chemotherapy may have side effects, including nausea, vomiting, hair loss, infection, and extreme fatigue. If the side effects of a regimen are too severe, the dose(s) or schedule(s) of the drug(s) may be modified, or the therapy may be discontinued altogether, and new medications can be used instead. In carefully selected patients, a very high dose of chemotherapy

(intravenous melphalan) is combined with stem cell transplantation. Stem cells are found in the bone marrow and they are the cells which develop into various types of normal blood cells including red blood cells and white blood cells. Once the abnormal plasma cells are destroyed using high doses of chemotherapy, the bone marrow is replenished with fresh stem cells previously collected from the patient's own body (this is called an "autologous transplant". The other type of stem cell transplant, an "allogeneic transplant," using a donor's cells, is not used to treat AL amyloidosis). Chemotherapy followed by stem cell transplantation often achieves an excellent response with significant improvement or stabilization of organ function. However, not all patients can tolerate this aggressive regimen, particularly those with advanced heart problems or significant autonomic dysfunction.

Another type of medicine being used to treat AL amyloidosis is monoclonal antibody therapy. These targeted proteins can be used in a couple of different ways.

First, there are commercially available antibodies which target the plasma cells and reduce them in a manner similar to chemotherapy. The one most widely used, and recently approved by the FDA, is daratumumab (Darzalex-Faspro). Daratumumab targets the CD38 molecule on the plasma cells. Daratumumab is approved to be given in combination with chemotherapy, but multiple studies have shown it to be effective when given by itself, as well. Other plasma cell-targeting antibodies currently being studied, either alone or in combination with chemotherapy, are isatuximab (Sarclisa) and elotuzumab (Empliciti). Newer antibodies which target plasma cells in different ways are being developed as therapy for multiple myeloma, and it is likely they will eventually be tested against AL amyloidosis, as well. A different way antibodies can potentially be used to treat AL amyloidosis is by using them to directly target the light chain amyloid

deposits which have accumulated in the body (rather than the cells making the abnormal light chains, the way daratumumab does). This is currently an experimental approach being studied in clinical trials. By targeting the amyloid deposits in this way, one's immune system can potentially identify, destabilize, and remove them more effectively.

AA Amyloidosis

AA (or secondary) amyloidosis is less common in developed countries now that treatments have been found for the many inflammatory conditions that can trigger this disease (e.g., rheumatoid arthritis, Crohn's disease, and familial Mediterranean fever). In AA amyloidosis, amyloid deposition is typically very gradual. The survival rate is often more than 10 years, particularly with treatment for kidney disease. In some instances, such as with untreated infections like osteomyelitis or tuberculosis, amyloid deposits may accumulate more rapidly. In all cases, the mainstay of therapy is to address the underlying infection or inflammatory condition. This slows or stops the progressive buildup of amyloid by reducing the circulating precursor protein, serum amyloid A. For those patients with renal failure, dialysis and kidney transplantation are possible treatments. Importantly, with kidney transplantation, if the abnormal protein which caused the original injury to the kidney is not addressed, amyloid may eventually appear in the donated kidney. This is true of all types of amyloidosis, not just AA amyloidosis.

ATTRv (Hereditary Amyloidosis)

In ATTRv amyloidosis, the heart and nervous system are most commonly affected. Without intervention, depending on the specific mutation, the survival rate ranges between 5-15 years from the onset of the symptoms of the disease.

For many years, since most of the transthyretin protein is produced in the liver, liver transplantation using either a cadaver liver or a sec-

tion of a healthy live donor's liver was the definitive treatment. Potential obstacles to liver transplant include the poor health status of many patients with ATTRv, and also the lack of available donor livers. More recently, with approved therapeutic treatments available, and new drugs under development, liver transplant is no longer a common treatment in the United States and many other countries.

These newer treatments focus on two general methods to prevent amyloid deposits forming from the misfolded TTR protein. One class of drugs works at reducing the amount of TTR made by the liver (gene silencers), and the other works at rendering the TTR unable to misfold into amyloid fibrils (stabilizers).

Evidence shows that by reducing the abnormal protein available to become amyloid, organ function can improve. Two gene silencers currently approved by the FDA for treatment are patisiran (Onpattro) and inotersen (Tegsedi). When these drugs bind with the target TTR mRNA that is used as a template to produce TTR protein, the mRNA is degraded by the cell and recycled, preventing it from being used for TTR protein production. Put simply, “no TTR mRNA = no TTR protein, and no TTR protein = no TTR amyloid formation”. While the goal is to stop progression of the disease with these drugs, some patients have reported improvement with their symptoms as well.

The first FDA approved stabilizer for ATTR patients is tafamidis (Vynamax/Vyndaqel). This drug prevents the mutant TTR from misfolding into amyloid. Another stabilizer in use is diflunisal (Dolobid; FDA approved for other indications, sometimes used off-label for ATTR). There are other stabilizers in clinical trials; acoramidis, and tolcapone being two of them. These oral drugs are small molecules that bind to the precursor proteins and stabilize their structure, so that they do not form amyloid fibrils and accumulate in the body.

As in AL amyloidosis, various antibody treatments are being studied that can directly target the amyloid deposits which have accumulated in the body. This is currently an experimental approach being studied in clinical trials. By targeting the amyloid deposits in this way, one's immune system can potentially identify, destabilize, and remove them more effectively.

ATTRwt (Wild Type Amyloidosis) (Formerly “Senile Systemic Amyloidosis or Age Related”)

In wild type transthyretin (ATTRwt) amyloidosis, the currently approved stabilizer, tafamidis (Vyndamax/Vyndaqel), is considered the standard of care. New stabilizers as well as the gene silencing drugs approved for the hereditary form of the disease are also in clinical trials for ATTRwt. In some of the more dire cases, when the patient meets strict criteria, heart transplantation may be considered.

A β_2 M Amyloidosis

In A β_2 M (or dialysis-related) amyloidosis, kidney transplantation is considered the best therapeutic option. Low copper dialysis membranes may prevent or delay onset of the disease.

Localized Amyloidosis

For amyloid deposits that occur in isolated areas, such as the bladder or airways, there are several approaches to slow down the disease's progression. Once a systemic disease has been ruled out, surgical removal, radiation, and laser treatments may be considered. In many of the more benign cases where quality of life is not an issue, treating the symptoms or no treatment at all might be recommended along with frequent checkups. As with all forms of amyloidosis, patients are encouraged to have periodic checkups to monitor their condition. Fortunately, most patients with localized amyloidosis do not go on to develop chemotherapy-requiring systemic amyloidosis. For cerebral amyloid angiopathy (CAA) which af-

fects the brain, there is no known effective treatment. The goal is to relieve symptoms. This can include medications that help to improve memory, such as those used to treat Alzheimer's disease. Seizures, sometimes called "amyloid spells," may be treated with anticonvulsants such as phenytoin (Dilantin) or carbamazepine (Tegretol). It is also important to limit the use of medications which can increase the risk of brain bleeding, such as aspirin or blood thinners. In some cases, speech and physical therapies are needed.

Treating the Symptoms of Amyloidosis

It is very important to treat not just the underlying causes of amyloidosis, but the symptoms of the disease as well. This will ensure a patient's quality of life and longevity.

Normal, everyday activities can be carried out if one is able. However, if fatigue or shortness of breath occurs, it may be necessary to rest. One should not exert him or herself beyond what is recommended by their doctor.

To address the symptoms related to amyloid involving the kidneys and heart, patients may need to take a diuretic drug to increase urine production, as prescribed by their doctors; limit the amount of salt in their diet; or wear elastic stockings and elevate their legs to lessen the swelling.

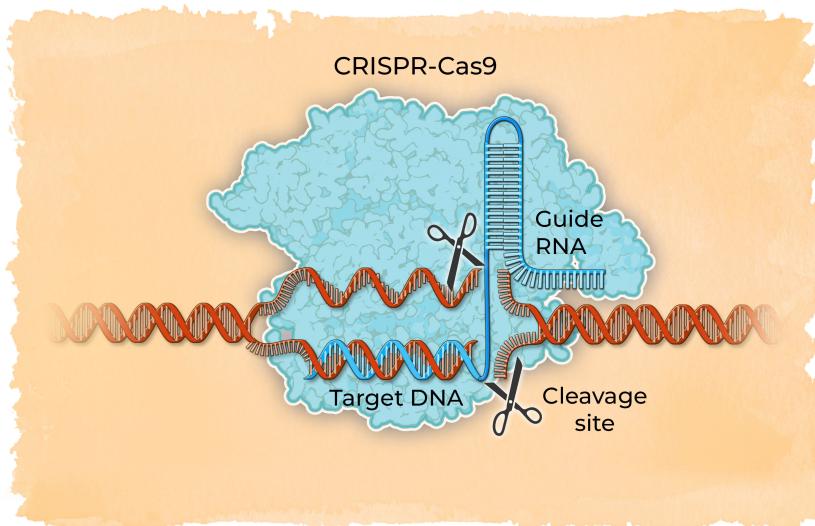
For the gastrointestinal tract, certain foods or medications can help with diarrhea and constipation. Sometimes dietary changes may help relieve symptoms or maintain body weight.

Although symptoms stemming from damage to the nerves (neuropathy) can improve with effective anti-amyloidosis therapy, it may take 12-24 months or longer for the nerves to recover. While waiting to determine if this improvement occurs, medications may be used to

alleviate pain. These may be taken orally or applied to the skin. For discomfort, tingling or burning, the use of a warmwater foot massager for 15 minutes before bed can help with sleeping. The warm water and vibrations stimulate non-pain transmitting nerves and block the pain-transmitting nerves.

Gene Editing (CRISPR)

Since some forms of amyloidosis are the result of a mutation in a gene contained within a person's DNA, one might wonder whether there is a way to directly target or edit the gene itself to fix the problem. Theoretically, this might have advantages over some of the previously discussed treatment approaches. While the "gene silencers" patisiran and inotersen largely shut down the production of TTR protein by degrading TTR mRNA, they do nothing to decrease the rate at which TTR mRNA is made from the TTR DNA gene template in the first place. This is why these medications (and others like tafamidis, which target the protein itself) need to be used on a continuous basis to be effective. However, if the DNA itself were permanently



Gene editing for ATTR amyloidosis currently in clinical trials

edited so that mRNA for a particular gene could no longer be made at all, there would be no need to continuously target downstream TTR mRNA or protein. Thanks to a recent scientific advance called CRISPR, this may soon be possible. CRISPR can be used to precisely edit genes and is such a major advance over older gene editing techniques that the inventors of CRISPR won the 2020 Nobel Prize in Chemistry. Currently there is ongoing research into the use of CRISPR as therapy for amyloidosis. While the advantages would appear to be obvious there must be more study to research any long-term effects of not having TTR as well as any “off target” effects that could cause serious adverse events.

Participating in Clinical Research

Clinical trials are research studies that test new ways to diagnose and treat disease. Such research is essential to improve our understanding of amyloidosis and to develop more effective therapies. The treatments that are available today were all developed and refined through this ongoing clinical research. Now patients can achieve durable, long-term remission of their disease, along with major organ system improvement.

For qualifying patients, there is an opportunity to participate in clinical trials. New treatments are tested in clinical trials to determine whether they are as good as, or better than, existing standard treatments. All proposed clinical trials must be approved and overseen by an Institutional Review Board (IRB). The IRB is comprised of physicians, scientists, and non-scientific members (which may include clergy or other lay people). They are there to ensure the safety of participants in clinical trials.

Participation in clinical trials is completely voluntary, and participating patients sign an informed consent form. It is also okay to withdraw from the trial at any time. In many cases, the cost of treatment may be covered as part of the study.

Being involved in clinical research potentially allows patients to benefit from new, experimental treatments before they are widely available. In the long run, this leads to improved medicines and therapies for everyone. To learn what clinical trials are currently recruiting, one may consult with the amyloidosis centers or visit ClinicalTrials.gov. Also, patients can search PubMed.gov to find scientific, peer-reviewed articles about research which has already been completed.



Early and accurate diagnosis, along with an individualized treatment plan, are key to achieving positive outcomes for patients and families. With an extended support community of healthcare providers and peers, you are not alone.

6. MAJOR AMYLOIDOSIS CENTERS

There are many qualified physicians to help with diagnosis and treatment of amyloidosis. As patients, you are not alone. In the United States, please contact the Amyloidosis Support Groups for 24-hour help and guidance. The toll-free number is (866) 404-7539, or email Info@AmyloidosisSupport.org. The following is a list of major research and treatment centers in the United States and internationally. A more complete list can be found at www.AmyloidosisSupport.org. Because amyloidosis varies with each case, the invaluable expertise of these centers will help to promote positive outcomes for patients and families.

U.S. Amyloidosis Centers

- Atrium Health Levine Cancer Institute (Charlotte, NC)
- Baylor University Medical Center/Texas Oncology (Dallas, TX)
- Boston University Medical Center (Boston, MA)
- Brigham and Women's/Harvard (Boston, MA)
- Cedars Sinai (Los Angeles, CA)
- City of Hope (Duarte, CA)
- Cleveland Clinic (Cleveland, OH and Weston, FL)
- Columbia University Irving Medical Center (New York, NY)
- Duke University (Durham, NC)
- Emory University - Winship Cancer Institute (Atlanta, GA)
- Froedtert & The Medical College of Wisconsin (Milwaukee, WI)
- Fred Hutchinson Cancer Center (Seattle, WA)
- Houston Methodist (Houston, TX)
- Indiana University (Indianapolis, IN)
- Johns Hopkins Hospital (Baltimore, MD)
- Karmanos Cancer Institute (Detroit, MI)
- Loyola University (Maywood, IL)
- Mayo Clinic (Rochester, MN; Jacksonville, FL; Phoenix, AZ)
- MD Anderson Cancer Center – (Houston, TX)
- Medical University of South Carolina (Charleston, SC)
- MedStar-Georgetown (Washington, D.C.)
- Memorial Sloan Kettering Cancer Center (New York, NY)
- Moffitt (Tampa, FL)
- Mount Sinai (New York, NY)
- Northwestern University (Chicago, IL)

- OhioHealth (Columbus, Ohio)
- Ohio State University Comprehensive Cancer Center, (Columbus, Ohio)
- Oregon Health and Science University (Portland, OR)
- Penn Medicine Abramson Cancer Center (Philadelphia, PA)
- Rochester Regional Health (Rochester, NY)
- Rush University Medical Center (Chicago, IL)
- Saint Luke's Hospital System (Kansas City, KS)
- Scripps (San Diego, CA)
- Stanford (Palo Alto, CA)
- Tufts Medical Center (Boston, MA)
- UAB Medicine (Birmingham, AL)
- UCSD - Moores Cancer Center (San Diego, CA)
- UCSF - Helen Diller Family Comprehensive Cancer Center (San Francisco, CA)
- University of Chicago (Chicago, IL)
- University of Kansas (Lawrence, KS)
- University of Miami - Sylvester Comprehensive Cancer Center (Miami, FL)
- University of North Carolina (Chapel Hill, NC)
- University of Rochester Medical Center-Wilmot Cancer Institute (Rochester, NY)
- University of Tennessee (Knoxville, TN)
- UT Southwestern Medical Center (Dallas, TX)
- University of Utah - Huntsman Cancer Institute (Salt Lake City, UT)
- Vanderbilt University Medical Center (Nashville, TN)
- Weill Cornell Medical Center (New York, NY)

International Amyloidosis Centers

- Monash University Eastern Health Clinical School, (Melbourne, Australia)
- Westmead Hospital (Sydney, Australia)
- Center for the Study of Familial Amyloidosis (Rio de Janeiro, Brazil)
- Cross Cancer Institute, University of Alberta, (Edmonton, Canada)
- Princess Margaret Cancer Centre (Toronto, Canada)
- University of British Columbia, (Vancouver, British Columbia, Canada).
- National Centre for Amyloidosis (London, England)
- Centre Hospitalier Universitaire & Reference Center for AL Amyloidosis, (Limoges, France)
- Amyloidosis Center University Hospital Heidelberg (Heidelberg, Germany)
- National and Kapodistrian University of Athens, (Athens, Greece)
- Amrita Amyloid Center (Kochi, India)
- Hadassah Medical Center, Faculty of Medicine, Hebrew University (Jerusalem, Israel)
- Center for the Study & Cure of Systemic Amyloidosis (Pavia, Italy)
- Kumamoto University Hospital (Kumamoto, Japan)
- University of Groningen, University Medical Center Groningen (The Netherlands)
- Oslo University Hospital (Oslo, Norway)
- Universidade do Porto (Porto, Portugal)
- Amyloidosis and Multiple Myeloma Unit, Department of Hematology, Hospital Clínic of Barcelona (Barcelona, Spain)

The list of amyloidosis centers is ever changing. Should you have any questions, or wish more information about a center not on the list, please email Info@AmyloidosisSupport.org

7. ONLINE RESOURCES

For more information, including local and virtual support meetings please visit:

Amyloidosis Support Groups

AmyloidosisSupport.org

Other helpful resources include:

- Amyloidosis Alliance
amyloidosisalliance.org
- Amyloidosis Foundation
amyloidosis.org
- Amyloidosis Research Consortium
arci.org
- Amyloidosis Speakers Bureau
mm713.org/speakers-bureau
- Amyloid Support Group UK
amyloidosis.org.uk
- ATTR Amyloidosis All Ireland Support Group
<https://www.facebook.com/groups/ATTRAmyloidosisIrelandSupportGroup>
- Canadian Amyloidosis Support Network Facebook Group
facebook.com/groups/194563300561853
- National Organization for Rare Disorders
rarediseases.org

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