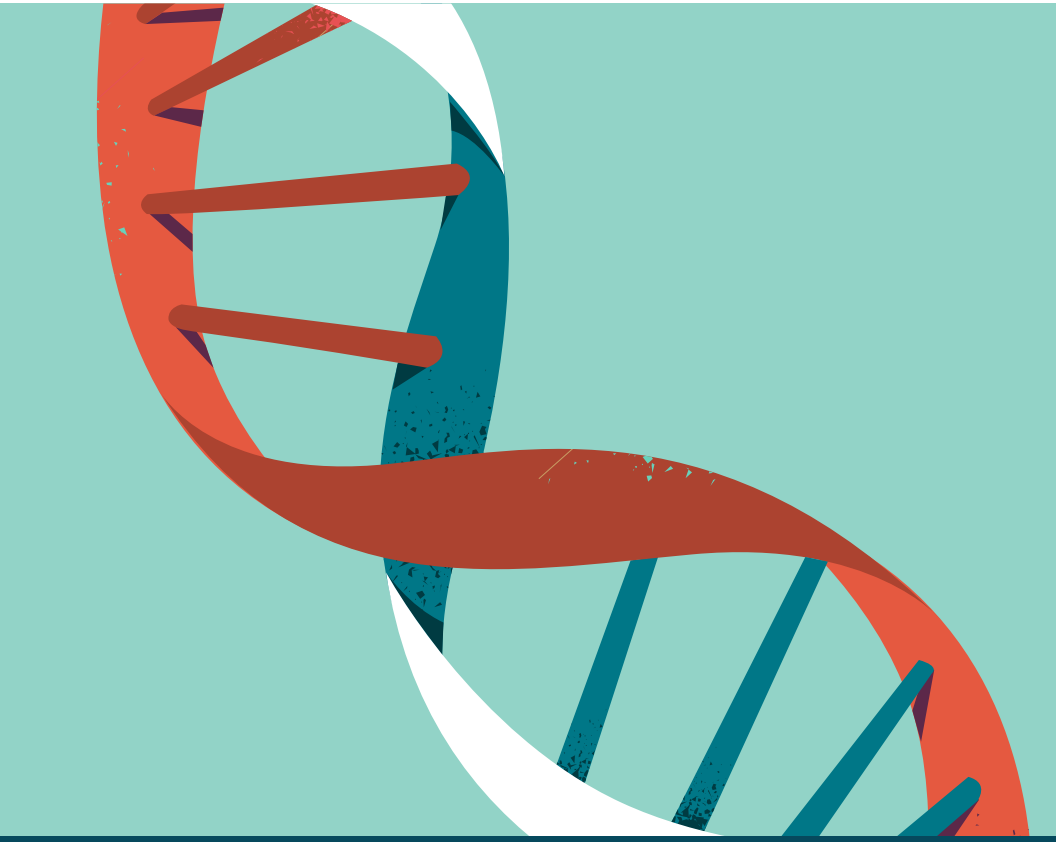
UNDERSTANDING GENETICS IN HEREDITARY TTR AMYLOIDOSIS



Amyloidosis Research Consortium

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KNOWLEDGE IS POWER

ABOUT THE AMYLOIDOSIS RESEARCH CONSORTIUM

The Amyloidosis Research Consortium (ARC) is a nonprofit organization dedicated to driving advances in the awareness, science, and treatment of amyloid diseases. Its mission is to improve and extend the lives of those with amyloidosis. ARC is committed to collaborative efforts that accelerate the pace of discovery, expand patient access to the most effective care, and improve short- and long-term outcomes. Working with partners in industry, government, and academia, ARC seeks to spark innovation and to bring promising treatments from labs to clinics. Its outreach and education inform and empower patients, families, caregivers, physicians, and researchers.

To learn more about ARC, visit **www.arci.org** or call **(617) 467-5170**.

Amyloidosis Research Consortium

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INTRODUCTION

ABOUT AMYLOIDOSIS

Amyloidosis is a group of diseases caused when misfolded proteins, called amyloid, build up and form fibrils that deposit in the body's organs and tissues, affecting their ability to function. In hereditary amyloidosis, a change (mutation) in a gene leads to the protein misfolding and forming amyloid fibrils. There are multiple forms of hereditary amyloidosis including:

- Transthyretin amyloidosis
- Apolipoprotein AI amyloidosis
- Apolipoprotein AII amyloidosis
- Gelsolin amyloidosis
- Lysozyme amyloidosis
- Fibrinogen Aa amyloidosis
- Cystatin C amyloidosis

The specific hereditary form someone has depends on which gene carries the mutation. More hereditary forms may be discovered in the future as research in this area continues.

The most common hereditary form is hereditary transthyretin amyloidosis (hATTR). This condition is due to a mutation in the TTR gene, which provides the instructions for the transthyretin protein. Transthyretin is mainly made in the liver, but it is also produced in the brain **(choroid plexus)** and eye, in smaller amounts. Transthyretin is involved in the transport of a thyroid hormone called **thyroxine**. Transthyretin also transports **retinol-binding protein**, which is responsible for transporting vitamin A around the body. When transthyretin misfolds it forms amyloid, and then the amyloid deposits in different organs and tissues throughout the body, most commonly in the heart and around the nerves. This can cause a variety of symptoms including fatigue, shortness of breath, numbness and tingling in the hands and feet, and/or carpal tunnel syndrome. Without treatment, these symptoms can significantly impact quality of life.

UNDERSTANDING GENETICS

Genes are the instruction manual for our body. We have more than 20,000 genes and each plays a different role. Some genes provide the instructions for specific traits like eye color or height, while other genes detail how the body should work. In general, 50% of our genes come from each parent. As a result, if a parent carries a genetic mutation, it is possible the mutation is passed on to one or more of their children.

A change or mutation in a gene can lead to a genetic condition. If you think of a gene as a sentence, a mutation is like a spelling mistake in the sentence. As a result, the body can no longer "read" the instructions. Mutations can be passed down from one generation to the next or occur spontaneously.

While genetic conditions are individually rare, in total they affect around 3.5% of the general population. There are over 10,000 different known conditions caused by mutation(s) in a gene. Knowing information regarding your genetics can provide valuable information to your healthcare team, including the likelihood of developing a condition or passing a gene on to any offspring, symptoms to be aware of, and possible age of disease onset. It can also help guide treatment, as specific medications have been approved for hereditary ATTR amyloidosis.

GENETICS IN HEREDITARY AMYLOIDOSIS

There are over 120 different mutations that have been identified in the TTR gene and as research continues, it is possible that more may be discovered. Each mutation causes a different pattern of disease onset, symptoms and outlook. The most common ATTR mutations are the:

- Val30Met (p.Val50Met) mutation – found mostly in those of Portuguese, Spanish, French, Swedish, and Japanese descent.
- Val122Ile (p.Val142Ile) mutation – Found in those of African descent. It is observed in 3-4% of African Americans in the US.
- Thr60Ala (p.Thr80Ala) mutation – seen in people of Irish descent and represents the most common mutation in the UK.

Globally, the most common mutation is p.Val30Met (p.Val50Met), and in the United States, the most common mutation is p.Val122Ile (p.Val142Ile), followed by the p.Thr60Ala (p.Thr80Ala) mutation.

Mutations can be common in certain areas of a country due to the founder effect, which occurs when a small subset of people, including mutation carriers, move to a new territory and create a population in which the mutation is concentrated. For instance, in the Appalachian mountains the p.Thr60Ala (p.Thr80Ala) mutation is common due to Irish immigrants settling there.

What's in a name?

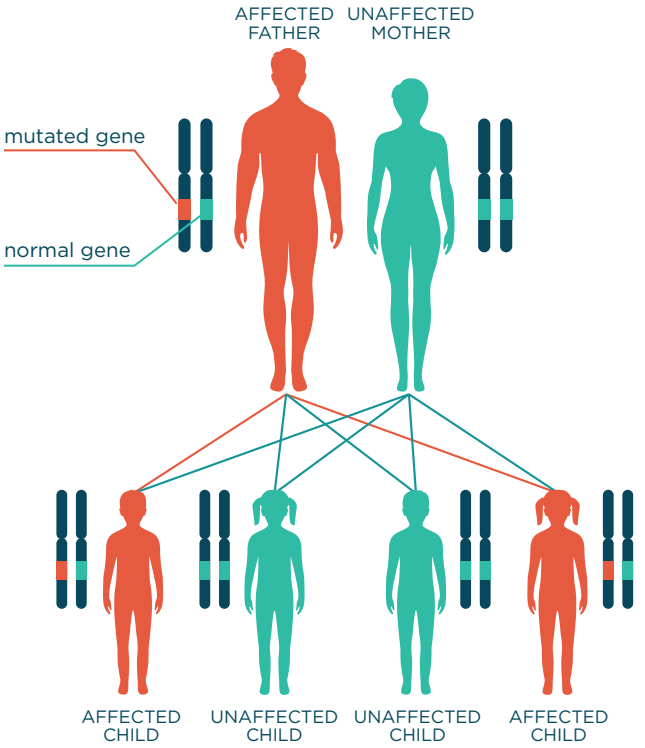
One may wonder why mutations may be listed with two different titles. This variation in the name of mutations reflects their understood position in the amino acid sequence chain. The amino acid chain is what all proteins are comprised of, including the ATTR protein. Previously, researchers utilized an amino acid chain sequence that did not include the first 20 amino acids. Over time, more and more researchers have begun to utilize the full amino acid chain sequence. Therefore, the mutation titles have changed to reflect their actual position in the amino acid chain sequence. In their titling, each mutation was moved further by 20 amino acids in the sequence, i.e. p.Val30Met -> p.Val50Met.

CHANCE OF INHERITANCE

Hereditary transthyretin amyloidosis (hATTR) is an **autosomal dominant** condition. This means inheriting only one copy of a mutated gene can cause the condition. Every person has two copies of the TTR gene, one from each parent, and in hereditary amyloidosis, there is a mutation in at least one of those copies. If a person carrying the mutated gene has children, there is a 50% chance of passing on the mutated gene. Therefore, all first-degree relatives (parents, children, and siblings) have a 50% chance to carry the mutation and be at an increased risk to develop

hereditary amyloidosis. In rare cases, an individual may carry two mutations (called **homozygous** or **compound heterozygous**), one in each copy of the TTR gene. In that case, the likelihood of other family members also carrying a mutation is higher.

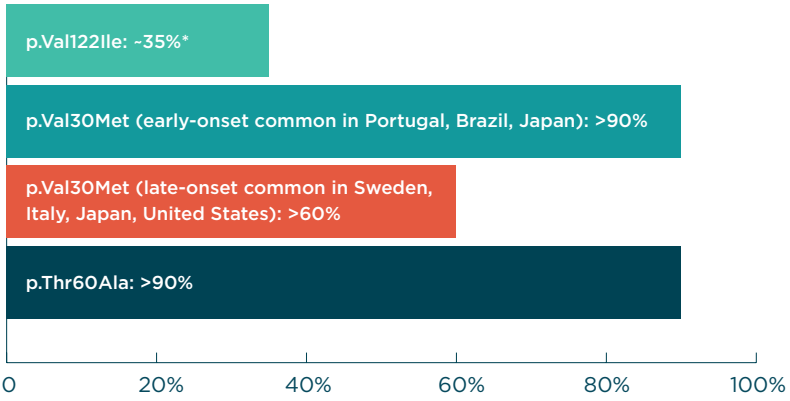
AUTOSOMAL DOMINANT INHERITANCE PATTERN



Although an individual may carry a mutation, it is not guaranteed they will develop hereditary amyloidosis. The term **penetrance** refers to the likelihood of a gene-carrier developing a disease or condition. In hereditary amyloidosis, penetrance depends on a variety of factors including the specific mutation, which parent carried the mutation, and where a carrier lives. For example, two individuals can carry the p.Val30Met mutation in TTR and the individual who lives in Portugal may develop symptoms in their 30s while someone who lives in Sweden may not develop

symptoms until their 60s. Research is currently being conducted to understand why this occurs.

PENETRANCE FOR COMMON MUTATIONS



*The penetrance rate for Val122Ile still remains unclear. The percentage listed above reflects its likeliness to occur by age 75.

Source: ACC Consensus Statement.

The age at which an individual typically starts to develop symptoms (age of onset) also partially depends on the specific genetic mutation and the family history. Knowing your family history can provide important clues as to when individuals in your family may develop this condition which can guide screening.

EFFECTS OF GENETICS ON FAMILY LIFE

Whether or not someone decides to have children is a personal decision and often depends on a variety of factors. For some people, one of those factors is the likelihood of passing on a hereditary condition such as hereditary amyloidosis. As discussed above, if someone carries a mutation in the TTR gene, there is a 50% chance each of their offspring would inherit the mutation and be at risk of developing amyloidosis in the future. Remember, if an individual inherits the mutation from a parent, it is not a guarantee they would develop amyloidosis.

In family planning, testing options include a prenatal genetic testing option which involves testing embryos for the mutation and selecting an embryo which does not carry the mutation to implant. This process requires using in vitro fertilization (IVF), where eggs and sperm are harvested, and the embryo is created in a laboratory. While insurance may cover IVF for infertility reasons, it typically will not cover the process for other indications making this procedure expensive. Whether or not this process is right for you and your family is a personal decision. There is no "right" answer.

WHAT IS GENETIC TESTING?

Genetic testing looks for mutations in DNA. There are multiple types of genetic tests, and the best test depends on the specific genetic condition(s) being evaluated. For hereditary amyloidosis, typically the most appropriate test is TTR gene sequencing. This may be done as part of a panel of many genes or as a single gene test. There are some genetic tests, such as the test offered through 23andMe, which will only look for a few common mutations. Consequently, this type of testing is not complete and often further testing (gene sequencing) may be recommended.

Genetic testing is performed on a blood sample, saliva sample, or cheek cells which are collected by a swab called a **buccal swab**. The sample will then be sent to the lab for analysis. If you think of the gene as a sentence, the test is like a spellchecker analyzing for any spelling mistakes. Typically results are available in 2-4 weeks.

THE ROLE OF GENETIC COUNSELING

Genetic counselors are advanced healthcare professionals specializing in human genetics and counseling. The role of a genetic counselor is to help patients and family members understand their genetics and provide guidance in making healthcare decisions. Individuals can see a genetic counselor at any point in their medical journey, but it is often recommended to see a genetic counselor before and after undergoing genetic testing.

PRE-TEST COUNSELING

The goal of a genetic counseling session is to learn more about hereditary amyloidosis and the role of genetics in disease presentation. Often a genetic counselor will review basic genetic concepts and explain how a mutation in the TTR gene may lead to the development of amyloidosis. Family members are also welcome to attend genetic counseling sessions. It can be helpful to have relatives attend not only because they may also be at risk to have or develop the condition, but also because having a second person there to ask questions and hear the information can be helpful.

As with any medical test, it's important to understand why a test is recommended and the implications of the possible results. A nuance specific to genetic tests is to also understand what the test results may mean for family members. Many patients believe a genetic test result is black and white (the mutation is found or not found), and while this is often true for amyloidosis, a small number of patients may receive an "uncertain result." This means a genetic variation is identified in the TTR gene, and it is not known if this mutation is disease-causing. It's important to be aware of this possible result prior to testing. The genetic counselor will also discuss the logistics of testing such as medical insurance coverage, how the testing is done, and which genes will be analyzed. Of note, often life insurance and long-term care insurance policies are legally able to use genetic test results to determine coverage. This topic will be covered during the genetic counseling appointment, but for more information on this subject,

please see “Insurance Protocol” (on page 14).

Anticipating possible emotional responses to the results prior to testing helps ensure informed consent. It can also help prepare an individual for the testing process. As discussed below, there can be a variety of reactions to both positive and negative results, and during the pre-test counseling session the genetic counselor can help patients and families anticipate these possible reactions.

POST-TEST COUNSELING

Beyond the review of a “positive” or “negative” result, discussion of the genetic test results with a genetic counselor will include information regarding the specific mutation an individual tests positive for. This may include where in the body the amyloid is likely to deposit, the age at which symptoms often develop, and the likelihood of family members, who also carry the mutation, going on to develop amyloidosis. In the rare case of an “uncertain” result, the genetic counselor can provide further details about what is known about the potential mutation and recommendations for any further evaluation or family testing to learn more about the likelihood it is disease-causing. For further specifics about the results and medical management, the genetic counselor will likely provide a referral to an amyloidosis center.

Post -test counseling may also include discussion of care plan options. There are medications available for hereditary amyloidosis patients with polyneuropathy and/or cardiac symptoms that may slow or stop the progression of the disease. Discussion during the genetic counseling session may include an overview of these treatment options and referral back to the treating physician(s) for further nuanced discussion. If asymptomatic, discussion may also include anticipatory guidance of where amyloid may deposit and what symptoms to expect, especially without treatment.

As reviewed above, hereditary amyloidosis is an autosomal dominant condition, and all first-degree relatives (parents, children, and siblings) have a 50% chance to also carry the mutation and be at risk to have or develop hereditary amyloidosis. Depending on your life-stage, if you are thinking about having children and are concerned about passing on the mutation, the

genetic counselor can discuss family planning options such as prenatal genetic testing including testing embryos for the familial mutation. This is called PGT-M.

TESTING CONSIDERATIONS

POTENTIAL CHALLENGES OF A POSITIVE RESULT

When someone receives a positive genetic test result for hATTR, this indicates they are a carrier or are at risk of developing the condition. It is important to note that not everyone who carries a mutation in the TTR gene will develop hereditary amyloidosis. Some individuals will carry the mutation but never develop the disease.

Due to the increased risk for amyloidosis, clinical screening is recommended for individuals who carry a mutation known to cause amyloidosis. Screening should begin 10 years prior to the age at which the youngest person in the family with hereditary amyloidosis began to develop signs or symptoms. For example, if the youngest person in the family with amyloidosis began to develop symptoms at age 60, health screenings for genetic carriers should start at or near age 50.

As noted above, certain mutations are typically associated with a particular organ involvement. As a result, clinical screenings may differ, depending on the genetic mutation and which symptoms family members have developed. For individuals at risk for cardiac amyloidosis, screening may include an echocardiogram (ECG), technetium pyrophosphate scan (PYP), and bloodwork. For individuals at risk for neurologic involvement, screening may include a neurological exam, nerve conduction studies, assessment using specific the Neurologic Impairment Score (NIS). Professional guidelines vary, with recommended screening frequencies between one and five years.

Currently, there are no FDA approved medications for asymptomatic genetic carriers. Knowledge of this may play a role in whether an individual decides to undergo genetic testing. While currently there is no FDA approved medication for asymptomatic carriers, clinical trials are expected to soon

become available. Additionally, there are treatment approaches carriers can consider.

Diflunisal is an anti-inflammatory medication that has been approved by the FDA for pain management in conditions such as arthritis. It has also been found to stabilize the TTR protein and, some physicians prescribe diflunisal as an off-label treatment for individuals who are either at risk or have a diagnosis of hereditary amyloidosis. Although the prescription of Diflunisal is based on expert opinion, small study outcomes, and clinical experience, it has yet to go through the rigorous testing needed to gain FDA approval. Limited data suggest green tea extract (EGCG) and turmeric may also help prevent the development of amyloid fibrils, although this has not been proven definitively. It is recommended to speak with a physician with expertise in amyloidosis, if interested in learning more about diflunisal or any additional alternative options.

DEPRESSION AND ANXIETY

Individuals may experience a wide variety of emotions when receiving a positive genetic test result. There is no “right way” to feel and even within the same family, members may process the information differently.

Individuals who have developed the symptoms of amyloidosis may already experience a wide range of emotions including:

- Relief at finally having an answer to “why” they developed the condition.
- Concern or guilt regarding the possibility their children inherited the mutation.
- Depression or anxiety about how the condition might progress, especially if they have seen other family members with the condition who were diagnosed in late-stages and/or before treatment was available.
- Hope at the possibility of having access to FDA approved medications for treatment of hereditary amyloidosis.

Individuals who are at risk of developing amyloidosis may also experience a variety of emotions, including those listed above, as well as:

- Stress and anxiety about when or if they will develop amyloidosis.
- Envy of family members who tested negative.
- Shock and/or denial of the accuracy of the testing results.

Given the complexity of emotions surrounding genetic test results and possibly a diagnosis of hereditary amyloidosis, many patients find it helpful to speak with a counselor or therapist to help process the information. It can be especially helpful to seek someone outside of the family to speak with given the hereditary nature of this condition since family members may also be processing their own test results or risks of developing amyloidosis.

POTENTIAL CHALLENGES OF A NEGATIVE RESULT

It's common to assume most would consider a negative test for the familial mutation to be joyous news, but it can also be accompanied by feelings of guilt for not inheriting the mutation. Such feelings may arise when those who have tested negative have to witness family members face the challenge of living with hereditary amyloidosis. This is called "**survivor's guilt**." This is not an uncommon feeling, although inherited genetics are solely due to chance.

Because a negative result may come with a variety of emotions, it is important to allow for space to process these feelings. If such feelings continue to persist, it is recommended to seek help from a professional.

While this is not limited to family members who test negative, it is not uncommon for those who test negative to become caregivers for family members who do develop hereditary amyloidosis. This may be due to their physical ability to help, but also healthy family members may feel obligated to take on this role due to survivor's guilt. Regardless of the underlying reason, being a caregiver can be challenging. Therefore, it is important for caregivers to also prioritize their own well-being. If possible, it is recommended for caregivers to share responsibility of their duties with additional family members to avoid burnout.

COMPLICATIONS OF TEST REFUSAL

Deciding whether to pursue genetic testing for hereditary amyloidosis is a personal choice. There is no one "right" answer, and it is not uncommon for some family members to decline testing. Reasons for declining testing may include concerns regarding it affecting life insurance, limited preventative treatment, or an individual's desire to not know this information until they are in an age range where they may develop amyloidosis. Overall, it's most important to share the information so family members can make an informed decision about testing.

If an individual has a family history of hereditary amyloidosis but decides not to undergo genetic testing to confirm whether they are a carrier, it is recommended for that person to undergo regular screening for amyloidosis, as if they have received a positive genetic test result. If a patient has been diagnosed with hereditary amyloidosis and declines genetic testing, this can limit medical care by limiting which medications a patient may have access to. It also limits information family members may have regarding their own risk of developing amyloidosis.

INSURANCE PROTOCOL

Prior to undergoing genetic testing, it is important to consider the possible insurance implications. In the United States, a federal law called the Genetic Information Nondiscrimination Act (GINA) prevents most employers or health insurance companies from using genetic test results to determine coverage or rates. However, this protection does not extend to life insurance or long-term care insurance. These companies can use genetic test information to determine rates and coverage. Of note, genetic test results do not affect existing policies. Due to concerns about insurance discrimination, some people will delay genetic testing until obtaining life insurance or long-term care insurance. If one has already been diagnosed with amyloidosis, the impact of genetic testing on the ability to obtain these insurances is likely minimal compared to someone undergoing predictive testing. Many health insurances cover both genetic counseling and genetic testing for amyloidosis. To verify this with an insurance company, a patient may request CPT codes from their healthcare provider. Of note, there are also pharmaceutical companies with

programs that will cover the cost of genetic testing and genetic counseling for patients and family members. The Amyloidosis Research Consortium (ARC) can help connect patients and potential hereditary amyloidosis carriers to such programs.

TIPS FOR DECISION MAKING

Deciding whether to undergo genetic testing is a complex decision and depends on a variety of factors. Genetic counselors can help weigh the different options; guiding people to make decisions that are best suited for them. Some common considerations include:

- Potential impact on life insurance and long-term care insurance
- Availability of preventative treatment
- An individual's age and likely age of onset
- If someone has signs or symptoms of hereditary amyloidosis
- An individual's personality and whether they would prefer to know regardless of preventative treatment availability.

If someone has been diagnosed with amyloidosis, their physician may recommend genetic testing to determine the subtype which will then guide management. Not knowing which specific subtype of amyloidosis an individual has could lead to mismanagement in some cases, so such testing is very important.

FAMILIAL RELATIONS

If you have been diagnosed with hereditary amyloidosis or have received a positive genetic test result and are known to be at risk of developing the condition, it is important to share this information with relatives. There is no one way to share this information, and each family dynamic is unique. If unsure of how to discuss results, it may be helpful to think about how the family has shared important information in the past, such as other medical diagnoses or changes in employment, and use this as a model. In some families there is one point person who often takes on the role of sharing the information and if that relative is told, they will ensure everyone else is aware. In other families, sharing at family gatherings such as the holidays or Sunday dinners is ideal. Another option which can be helpful for those who are not

in close contact with relatives is to send a letter or email.

It's important to remember, the decision of whether to pursue genetic testing is a personal one. Loved ones may make a different decision than what is recommended by family members who have tested, and that is okay. The most important thing is to share awareness, so that family members can make an informed decision.

HOW TO GET GENETIC TESTING

If interested in genetic testing for amyloidosis, it is recommended to meet with a genetic counselor or a healthcare professional with expertise in the genetics of hereditary amyloidosis, prior to undergoing testing. You can locate genetic counselors near you by visiting **findageneticcounselor.nsgc.org**. These visits can often be done by telemedicine or in-person.

The genetic counselor or healthcare provider will ensure the correct genetic test is ordered as there are many different labs which offer genetic testing for amyloidosis. In most cases, it is recommended to use a medical genetic testing lab (CLIA certified) rather than a company such as 23andMe. If you are pursuing genetic testing due to a family history of the condition, you will want to bring a copy of your relative's positive genetic test results to the appointment, if possible, as this will guide your testing.

ARC can help you find clinicians that specialize in the diagnosis and treatment of hereditary amyloidosis. You can email us at support@arci.org or give us a call at (617) 467-5170.

MAP | My Amyloidosis Pathfinder

New trials are always in development to help expand treatment options and improve quality of life. Join MAP to receive notifications as new clinical trials and treatment centers are posted.

Treatment Center Selector

Clinical Trial Finder

www.myamyloidosispathfinder.org

GLOSSARY

Autosomal dominance. In the instance of passing a mutation down to an offspring, only one parent needs to have a mutation to pass it on, rather than two.

Buccal swab. A non-invasive way to collect DNA samples for testing. Buccal cells are found in the inner cheek and in the mouth.

Choroid Plexus. A system of blood vessels and cells in the fluid-filled spaces of the brain.

Diflunisal. An anti-inflammatory medication that has been FDA approved to treat conditions such as arthritis. Diflunisal has been found to stabilize TTR and is used as an off-label treatment for ATTR amyloidosis.

Echocardiogram (ECG). An ultrasound of the heart that shows how well it is working.

Founder Effect. The reduced genetic diversity which results when a population is descended from a small number of colonizing ancestors.

Genes. The basic unit of inheritance.

Genetic Information Nondiscrimination Act (Gina). An act to prohibit discrimination on the basis of genetic information with respect to health insurance and employment.

Homozygous/compound heterozygous. Occurs when an individual carries two mutations, one in each copy of the TTR gene.

In vitro fertilization (IVF). A process of fertilization where an egg is combined with sperm in vitro.

Nerve conduction studies. Measures how fast an electrical impulse moves through your nerve.

Neurologic impairment score (Nis). A scale to measure impairment in the context of a broad range of neurological conditions.

Penetrance. The likelihood of a gene-carrier developing a condition or the proportion of people with a particular genetic variant (or gene mutation) who exhibit signs and symptoms of a genetic disorder.

Pgt-M. A test used for single gene disorders only. It involves screening IVF embryos to determine If they carry a particular gene.

Retinol-binding protein. A carrier protein that binds retinol and transports vitamin A from the liver to the rest of the body.

Survivor's guilt. Guilt experienced when an individual survives a traumatic event or situation that others did not.

Technetium pyrophosphate scan. An Imaging scan used to detect cardiac involvement in amyloidosis.

Thyroxine. A hormone secreted into the bloodstream, made by the thyroid gland.

ARC PATIENT & CAREGIVER RESOURCES

The Amyloidosis Research Consortium (ARC) is a global organization with a mission to advance scientific discovery, improve access to state-of-the-art care, and empower patients with innovative educational tools and support. Companion booklets in our information series have been developed to inform and guide you: **arci.org/booklets**

Questions?
Email: **support@arci.org**

SUPPORT ARC

Help support ARC's effort to extend and improve the lives of patients with amyloidosis. Contact Carli Good, Director of Development, to donate today.

Email: **cgood@arci.org**

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