Hereditary ATTR Thr60Ala Amyloidosis

**Introduction**

Hereditary ATTR amyloidosis is an inherited genetic disease which variably affects the nervous system and the heart. It is also known as familial amyloid polyneuropathy (FAP) or familial amyloid cardiomyopathy (FAC). The commonest form of this disease seen in the UK is called ATTR Thr60Ala (or T60A) amyloidosis, or Thr60Ala FAP. It occurs most frequently in people with Irish ancestry. Symptoms usually appear between ages 45 and 80, most often after age 60. It is slightly more common in men than in women.

Until recently most people with ATTR amyloidosis affecting the heart, so called amyloid cardiomyopathy, were probably misdiagnosed as having heart disease due to other, more common causes, such as high blood pressure or ischaemic heart disease.

Recent development of new, sophisticated heart scans has helped doctors to diagnose amyloid cardiomyopathy and manage patients appropriately. The new types of scans are called cardiac MRI and DPD scans. They are discussed in Appendix 2 at the end of this information leaflet. Since these new scans have been available doctors have realised that amyloid cardiomyopathy is more common than was previously believed. But nobody knows just how common this condition is, and how frequently patients are still misdiagnosed.

Doctors at the National Amyloidosis Centre (NAC) at the Royal Free Hospital in Hampstead, London pioneered the use of these new scans for diagnosing amyloidosis in the heart. All patients in the UK

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**What causes ATTR Thr60Ala amyloidosis?**

This condition is caused by a mutation in the TTR gene which results in the production of an abnormal (‘variant’) TTR protein called Thr60Ala, sometimes called T60A. A mutation is a permanent change in the sequence of DNA which makes up the genes in all cells in the body. The DNA acts like a blueprint or recipe for building the proteins that make up the body. The proteins are made up of strings of amino acids, assembled in a precise order. The DNA determines the order in which amino acids are assembled. In people with the Thr60Ala mutation, an amino acid called threonine is replaced by an amino acid called alanine at position number 60 in the TTR molecule. Thus, the TTR molecule produced by the gene containing this mutation differs in this slight respect from normal, ‘wild-type’ TTR by just one amino acid among the 127 which form the whole protein. This different, ‘variant’, TTR is more amyloidogenic than the normal ‘wild-type’ TTR, meaning that it has a greater tendency to form amyloid fibrils which deposit in the tissues of the heart and in the nerves.
with a diagnosis of amyloid cardiomyopathy should be referred to the NAC for an assessment. This will allow patients to benefit from specialist expertise, and help with ongoing investigations to determine how common the condition really is.

If you or your relative have suspected or diagnosed amyloid cardiomyopathy or neuropathy, you can request a referral to the National Amyloidosis Centre at the Royal Free Hospital. The consultants at the NAC also welcome inquiries from relatives of patients with hereditary ATTR amyloid cardiomyopathy. Please contact Dr Julian Gillmore by email at j.gillmore@ucl.ac.uk.

**What is ATTR amyloidosis?**

Amyloidosis is a rare disease caused by abnormal deposition and accumulation of proteins in the tissues of the body. Amyloid deposits are composed mostly of protein fibres known as amyloid fibrils. These amyloid fibrils are formed when normally soluble body proteins aggregate (clump together) and then remain in the tissues instead of being safely cleared away. About 30 different proteins are known to form amyloid deposits in humans. These amyloid forming proteins are known as ‘precursor proteins.’ Amyloid deposits cause disease by gradually accumulating within organs and thereby disrupting the structure and damaging the function of the affected tissues.

Different types of amyloidosis are named according to the precursor proteins which form the amyloid fibrils. All have the initial ‘A’, denoting amyloidosis, followed by one or more letters identifying the particular precursor protein which forms amyloid fibrils within the amyloid deposits.

In ATTR amyloidosis, a protein called transthyretin (TTR) is the amyloid precursor protein that forms the amyloid deposits. Transthyretin (TTR) is a normal blood protein, mostly made in the liver and present in everybody. In healthy people, normal, so-called ‘wild-type’ TTR functions as a transporter of thyroid hormone and vitamin A (retinol) within the bloodstream, hence the name: ‘trans-thy-retin’.

There are three categories of ATTR amyloidosis:

1. Familial amyloid cardiomyopathy (FAC) (hereditary – runs in families and can overlap with FAP).
2. Familial amyloid polyneuropathy (FAP) (hereditary – runs in families and can overlap with FAC).
3. Senile systemic amyloidosis (not hereditary – does not run in families or cause polyneuropathy). This has recently been renamed as wild-type ATTR amyloidosis.

The hereditary types of ATTR amyloidosis affect people with genetic alterations (mutations) in the TTR gene. People with these mutations have structurally abnormal, amyloid-forming (amyloidogenic), ‘variant’ TTR in their blood, which clumps together and forms amyloid deposits in tissues. They nearly always also have normal, ‘wild-type’ TTR as well and this also deposits in the tissues in the process triggered by the abnormal variant.

People with the Thr60Ala variant often have amyloid deposits in their hearts (cardiomyopathy) and also in their nerves (neuropathy), so ATTR Thr60Ala amyloidosis may be categorised as either familial amyloid cardiomyopathy or a type of familial amyloid polyneuropathy.

People with wild-type ATTR amyloidosis have only the normal, ‘wild-type’ TTR in their blood, and do not have any abnormal ‘variant’ TTR. However even wild-type TTR is inherently amyloidogenic and in wild-type ATTR amyloidosis the normal protein clumps together and forms amyloid deposits.

This information leaflet is focused on ATTR amyloidosis associated with the Thr60Ala mutation. Other information leaflets available from the National Amyloidosis Centre discuss the other types of ATTR amyloidosis.

**Who gets ATTR Thr60Ala amyloidosis?**

The genetic mutation causing ATTR Thr60Ala amyloidosis was first identified in an Irish family in 1986. A cluster of cases have been identified in the County Donegal region of North West Ireland, where it has been estimated that 1% of the population have this mutation. Cases have been described all around the world, but most UK patients with this condition have some Irish ancestry.
Inheritance of ATTR Thr60Ala amyloidosis is autosomal dominant, meaning that affected patients have a 50% chance of passing the condition on to each of their children. However, the mutation has variable penetrance. This means that carrying this mutation does not guarantee that a person will develop amyloid cardiomyopathy. Some people carry the mutation all their lives but do not develop amyloid. Less than 40% of patients diagnosed with this condition have a definite family history of amyloidosis. Genetic testing in a healthy person without symptoms can provide information on whether the mutation is present, but cannot predict whether the person will go on to develop amyloidosis. For more information on genetics, see Appendix 1 at the end of this information leaflet.

How can you help us understand this condition better?

Many aspects of this condition are not fully understood, including its prevalence in the UK. If you or your family member have heart disease which is suspected to be hereditary amyloidosis, please contact Dr Julian Gillmore by email at j.gillmore@ucl.ac.uk to discuss the possibility of genetic testing. Genetic testing is performed using blood samples taken from a vein. Your involvement is critical to help us to learn more about the disease, so that we may be better able to treat patients.

Symptoms of variant ATTR Thr60Ala amyloidosis

Most patients with variant ATTR Thr60Ala amyloidosis have symptoms resulting from amyloid deposits in the heart and the nerves.

Around 40% of patients first come to medical attention because of symptoms of heart disease, caused by amyloid deposits in the heart. In around 60% of patients the first symptoms are due to amyloid deposits in the nerves. In these patients, cardiac investigations after diagnosis usually show that there are also amyloid deposits in the heart.

Amyloid cardiomyopathy

ATTR amyloid deposits in the heart muscle may cause no symptoms at all if they are small. But when amyloid deposits in the heart are large, they can lead to stiffening of the heart muscle. This is called a ‘restrictive cardiomyopathy’. When the heart muscle is stiff, the heart is unable to pump the blood around the body as efficiently as usual. Symptoms of heart failure may then appear, including:

- shortness of breath, sometimes just after mild exertion
- palpitations and abnormal heart rhythms, most frequently atrial fibrillation
- leg swelling (oedema)
- weight loss
- nausea
- fatigue and muscle weakness
- dizziness and collapse (syncope or fainting), which may occur after exertion, or after eating
- disrupted sleep
- angina (chest pain)

Breathlessness may be worse during exercise or when lying flat at night. Patients may feel more comfortable at night propped up with several pillows.

Neuropathy

The autonomic nerves are usually affected in this condition. Autonomic nerves control several automatic body functions, such as blood pressure and digestive system function. When there are amyloid deposits in these nerves the resulting condition is called autonomic neuropathy. Symptoms include:

- postural hypotension – fall in blood pressure on standing up which may cause dizziness or fainting
- diarrhoea and/or constipation, sometimes alternating
- upper gastrointestinal symptoms:
  - early satiety (feeling of fullness after eating little)
  - dyspepsia (indigestion)
  - difficulty swallowing
  - vomiting
- urinary retention
- impotence

In addition, there may be peripheral neuropathy, where amyloid deposits affect the nerves supplying sensation and movement to the hands and feet. Symptoms include numbness, tingling and pins and needles in the hands and feet. There may be carpal tunnel syndrome. Less
than a quarter of patients have peripheral neuropathy when they first become ill, and the symptoms usually remain fairly steady, without significant worsening.

**Diagnosis**

Patients diagnosed with variant ATTR Thr60Ala amyloidosis at the NAC are often referred by cardiologists or neurologists. Cardiologists may first suspect amyloid cardiomyopathy when a patient experiences symptoms of heart failure, as described above, and ECG, echocardiogram and sometimes cardiac magnetic resonance (CMR) findings are suggestive of amyloid deposits in the heart.

A heart biopsy may be recommended to confirm the presence of amyloid and distinguish between hereditary ATTR amyloid cardiomyopathy and AL amyloidosis affecting the heart. The distinction is very important, as treatment for these conditions is completely different. Sometimes nerve biopsy may also be used to diagnose this type of amyloidosis.

Genetic tests performed on blood samples taken from the patient’s vein can identify patients with TTR Thr60Ala amyloidosis.

The ‘gold standard’ test (the best available method) for diagnosing this condition is a combination of detection of ATTR amyloid on heart, gastrointestinal tract or nerve biopsy and genetic testing showing the Thr60Ala mutation in the TTR gene.

Biopsies from other parts of the body, such as abdominal fat or the rectum are often used to diagnose other types of amyloidosis. In variant ATTR Thr60Ala amyloidosis these tests can be useful if they do show amyloid. But in many patients with this condition these tests are negative despite the presence of amyloid deposits in the heart and nerves.

DPD scans, which are performed at the NAC, are very helpful for confirming the presence and amount of amyloid deposits in the heart and the ‘pattern of uptake’ may strongly suggest the ATTR type of amyloidosis. Cardiac magnetic resonance (CMR) scanning, also available at the NAC, can give additional important information. Recent advances in CMR technology have led to a dramatic increase in the frequency of detection of amyloid deposits in the heart.

The routine SAP scan, which is used in the NAC to identify amyloid deposits in many parts of the body, does not detect amyloid in the heart.

Special blood tests for so called cardiac biomarkers are frequently abnormal in hereditary amyloid cardiomyopathy. For more information, see Appendix 2 at the end of this information leaflet.

**Which tests can help to diagnose amyloid cardiomyopathy?**

- ECG
- Echocardiogram
- Cardiac magnetic resonance (CMR) scan
- Radionuclide imaging (DPD scan)
- Blood tests: “cardiac biomarkers” (NT-proBNP and troponin)
- Genetic tests
- Heart biopsy / nerve biopsy
- Abdominal fat biopsy / rectal biopsy

*For more details on each of these tests, see Appendix 2 at the end of this information sheet*

**Treatment**

Treatment of ATTR Thr60Ala amyloidosis is symptomatic and supportive for the majority of patients. Many standard medications used for heart failure are not effective for patients with amyloid deposits in the heart, but the following measures can be very helpful in managing the heart disease:

**Fluid balance**

Patients with cardiac amyloidosis should limit their fluid intake.

The most important principle of treatment for cardiac amyloidosis is strict fluid balance control. Specialist heart failure nurse involvement may be valuable.
When there is cardiac amyloidosis, the heart is often too stiff to pump the blood efficiently. This may lead to fluid build-up, causing leg swelling (oedema) and breathlessness due to fluid in the lungs. This problem is exacerbated if the patient drinks too much fluid.

There may be episodes of worsening symptoms if there is even a small fluid excess. It is preferable to catch and treat heart failure symptoms at an early stage, before they become overwhelming.

Fluid excess can be avoided by careful attention to the 3 Ds:

1. **Diet**
   
   Fluid intake should be steady and should usually not exceed **1.5 litres per day**.

   Salt intake should be strictly limited. Salt should not be added to food. In addition, this includes attention not just to salt added to the food from the salt pot, but also to food with high salt content such as crisps, bacon, canned meats, sausages, canned soups and smoked fish. It can be very helpful to meet with a dietician for precise and personalised dietary advice.

   If salt and fluid intake is too high, the benefits of diuretic drugs can be completely lost.

2. **Diuretics**

   Doctors may prescribe diuretics (water tablets) which help the body to clear excess salt and water via the urine.

   Taking these drugs is not a substitute for avoidance of excessive dietary salt and water. If patients consume too much salt and drink too much fluid, the benefits of diuretics may be lost.

   Removal of excess fluid from the body leads to reduced ankle swelling and breathlessness. The first diuretic prescribed is often furosemide. Other diuretics such as spironolactone may be added later to increase the effect. Diuretics lead to increased amounts of urine and should be taken first thing in the morning, sometimes with an additional lunchtime dose. It is important to follow the doctor’s advice carefully regarding diuretic dose and the time of day when the tablet should be taken.

3. **Daily weights**

   Patients should monitor and record their weight daily. Weight should be measured consistently, using the same scales, at the same time of day, usually first thing in the morning after passing urine, just wearing underwear.

   Each litre of excess fluid weighs one kilogram. Several litres of fluid can accumulate in the body without it being very noticeable. But an increase in weight can be an early warning sign of fluid retention.

   Variations from week to week or even from day to day of about ± 1-2 kilograms are normal. If there is greater variation in weight, the doctor or nurse treating the patient can recommend appropriate measures. These may include increasing or reducing the diuretic dose. Some patients learn over time how to make appropriate small adjustments to the diuretic dose according to their weight fluctuations.

   **The aim is to detect and treat any excess fluid, before the patient feels unwell because of the fluid overload.**

   Support stockings can be helpful for patients with leg oedema (swelling in ankles and lower legs).

   Monitor your weight and blood pressure daily. It is helpful if you record them in a chart that you can show your local heart failure team during regular reviews. It is important for your local cardiology/haematology teams to agree to an exact, pre-determined plan for monitoring your fluid balance and adjusting diuretic doses in the event of unexpected fluid retention.

**Medications**

Many standard heart failure medications reduce the already low blood pressure in patients with cardiac amyloidosis, and can actually worsen the symptoms. The following drugs should be used with caution:

- calcium channel blockers
- digoxin
- beta blockers
- angiotensin converting enzyme (ACE) inhibitors
- angiotensin receptor blockers
Under certain circumstances some other treatments may be helpful. For example:

- Alpha agonist drugs such as midodrine may help to maintain blood pressure and allow higher diuretic doses.
- In some cases anticoagulation may be recommended.
- A pacemaker may be recommended if there is a slow or irregular heart rate.
- An implantable cardioverter defibrillator (ICD) may occasionally be recommended if there is an abnormally fast heart rate leading to dizziness or loss of consciousness.

**Exercise**

It is believed that limited, light exercise is beneficial for the general well-being of patients with amyloid in the heart. However, it is important to be careful, and to exercise within limits, to avoid exhaustion. If there are symptoms on exercise, patients should not push themselves.

**Neuropathy**

If there is orthostatic hypotension, elastic stockings may be recommended. Drug treatment with midodrine may also be helpful. Care should be taken to avoid dehydration if there is vomiting and diarrhoea. Intravenous fluids and anti-nausea drugs may be necessary. There are drugs that can help to control diarrhoea and constipation, and others that can help to combat erectile dysfunction. Medications that may help to alleviate neuropathic pain include gabapentin, pregabalin and duloxetine. Medical staff can give advice regarding appropriate foot care and footwear. This is important in order to prevent painless ulcers at pressure points and to protect areas of the foot that lack sensation.

**Liver transplantation**

Most TTR in the body is produced in the liver, and liver transplantation has been helpful in some patients with familial amyloid polyneuropathy caused by mutations other than Thr60Ala. However, liver transplantation does not prevent the progression of cardiac ATTR amyloid deposits in Thr60Ala amyloidosis. When patients with this condition have undergone liver transplantation, normal ‘wild-type’ ATTR from the transplanted liver has formed new amyloid deposits in the heart, that build up over the template of the pre-existing ATTR Thr60Ala amyloid deposits. Liver transplantation is therefore not effective for patients with ATTR Thr60Ala.

**Heart transplantation**

Most patients with hereditary amyloid cardiomyopathy are too elderly to undergo a heart transplant. The risk of complications from this major operation is high with advanced age. Heart transplantation may very rarely be an option for a patient who presents with the disease at an unusually young age, before age 60.

**Drug interactions**

It is very important that patients tell their doctors about any drugs they may be taking, including complementary or alternative medications or supplements. Some drugs may interact inside the body and lead either to toxicity due to raised drug levels, or lack of effect due to reduced drug levels.

If patients with amyloidosis become ill or require any treatment for a different condition it is important to inform the treating doctors so that, if necessary, the NAC doctors can be informed, in order to maintain co-ordinated care.

**Surgery and anaesthesia**

It is generally advisable for patients with amyloidosis to avoid undergoing surgery, anaesthesia and other invasive procedures.

If such procedures are necessary, patients should request that the surgeons, anaesthetists and other doctors involved contact the NAC doctors beforehand, to discuss any special considerations. For example, it is very important that great care is taken to monitor and maintain blood pressure and fluid balance throughout such procedures. Care should also be taken because of the tendency of tissues with amyloid in them to bleed and to heal poorly.

**Outlook**

Hereditary amyloid cardiomyopathy is a disease which progresses slowly. Patients with this condition survive longer than those with AL amyloidosis in the heart. Most patients with hereditary ATTR Thr60Ala amyloidosis survive for over 6 years after symptoms start.

Furthermore, a number of new drugs for ATTR amyloidosis are in various stages of development. These drugs are not yet available, but they do offer hope for the future.
New drugs in development for ATTR amyloidosis

**Diflunisal**

This belongs to a class of drugs called ‘non-steroidal anti-inflammatory drugs’ (NSAIDs). Drugs from this class are in common use as pain killers, for conditions such as arthritis. Diflunisal is bound by TTR in the blood. This binding is thought to make TTR less amyloidogenic. Trials are currently underway to assess the effect of diflunisal on the progression of neuropathy and cardiomyopathy in patients with familial amyloid polyneuropathy and familial amyloid cardiomyopathy. The first study report was recently published, with an encouraging result, but the numbers of patients involved was small and the extent of benefit was modest. The trial involved 130 patients with familial amyloid polyneuropathy (hereditary ATTR amyloidosis which affects the nerves), 64 of whom received diflunisal for 2 years while 66 received placebo (dummy pills). The rate of progression of neuropathy was slower in the patients who received diflunisal than in those who did not. Results of trials of diflunisal in cardiac ATTR amyloidosis are not yet available. It is important to note that NSAIDs such as diflunisal may have serious side effects, which may be especially dangerous in patients who are already unwell with amyloidosis. These side effects include:

- bleeding from the stomach and gut
- worsening of kidney function
- worsening of heart failure

Diflunisal use for ATTR amyloidosis is an ‘off-label’ indication, and only amyloidosis specialists should prescribe it.

**Tafamidis**

Tafamidis was developed as a specific drug for ATTR amyloidosis. It is bound by TTR in the blood. This binding is thought to stabilise the TTR and make it less amyloidogenic. Tafamidis has been studied in a trial involving 91 patients with early familial amyloidosis polyneuropathy (hereditary ATTR amyloidosis which affects the nerves). Progression of neuropathy was slightly slower in patients who received the drug than in those who did not. However, the difference was not statistically significant. Given the major clinical unmet need, tafamidis has been approved in Europe, but only for early stage neuropathy caused by hereditary ATTR amyloidosis. Since the evidence that tafamidis has a beneficial effect on polyneuropathy is not strong, it has not been approved by the FDA in the USA or by NICE in the UK. Tafamidis has not received approval for cardiac ATTR amyloidosis.

Other therapies that are currently in early stages of development and clinical trials include:

**Genetic based therapies**

- small interfering RNA
- antisense oligonucleotides

These two approaches aim to “switch off” the gene for TTR in liver cells, so that TTR is simply not produced.

A drug called ALN-TTRsc, which belongs to the small interfering RNA drug class, was shown to reduce blood levels of TTR by up to 94% in healthy volunteers. ALN-TTRsc is currently undergoing preliminary clinical trials in patients with ATTR cardiac amyloidosis, both senile systemic amyloidosis (recently renamed as wild-type ATTR amyloidosis) and the inherited forms of ATTR cardiac amyloidosis.

Another drug called IONIS-TTRRx belongs to the antisense oligonucleotide drug class. IONIS-TTRRx is currently undergoing trials in patients with familial amyloid polyneuropathy, a hereditary type of TTR amyloidosis. Current trials are only assessing the impact of this drug on nerve damage caused by TTR amyloid.

**Antibody mediated amyloid elimination**

Serum amyloid P component (SAP) is a normal blood protein, present in everybody, which is always present in amyloid deposits of all types because it binds strongly to all amyloid fibrils. The Wolfson Drug Discovery Unit has developed a drug called CPHPC, which clears all the SAP from the blood but leaves some SAP bound to the amyloid deposits. After CPHPC has been administered, it is therefore safe and feasible to administer antibodies to SAP which target the amyloid.

In experimental models these antibodies trigger the body’s normally very efficient systems for removal of debris from tissues to act on the amyloid. This approach works very well in models and there were encouraging results in the first, early stage trial in patients with systemic amyloidosis, conducted in collaboration with GlaxoSmithKline. A further trial, specifically focussed on cardiac amyloidosis, is currently being planned. If it proves to be safe and effective in humans, it will be applicable to patients with ATTR amyloidosis.
Basic genetics - understanding inheritance

The human body is made up of millions of tiny cells, each of which contains identical copies of the genes which we inherit from our parents. These genes function like an instruction manual, or a recipe book for the cells to construct the proteins and other substances which make up the body. Human cells each contain about 25,000 different genes.

Each cell contains two copies of each gene, one from each of our parents. Within each cell, the genes are arranged into forty six long strings, called chromosomes. Twenty three chromosomes come from the father and twenty three from the mother. Complicated interactions between the two copies of each gene determine how the body is composed, inside and out. External traits, like hair colour, eye colour and height and internal traits like blood group are all a consequence of which genes we inherit from our parents. The sex chromosomes determine whether a person is a man or a woman. Women have two X chromosomes and men have one X and one Y chromosome in every cell of their bodies. The illustration above shows the chromosomes from the cell of a man.

When a gene is located within one of the sex chromosomes, the way in which it is inherited is called ‘sex-linked’. Diseases that result from a mutation (abnormality) in a gene within a sex chromosome may be passed from parent to child by ‘sex-linked’ inheritance.

ATTR Thr60Ala amyloidosis is inherited by autosomal dominant inheritance, discussed below.

How do mutations cause amyloidosis?

The genes act like an instruction manual or a recipe for protein production inside every cell of the body. Sometimes an alteration or error may arise within a gene. This is called a mutation.

Anyone who has ever baked a cake knows that a single error in the recipe may have a number of different effects on the final product. It may lead to complete disaster, for example if you put in salt instead of sugar, or forgot the baking powder. Alternatively, there may be little effect on the final product, for example if you used margarine instead of butter.

Similarly, a mutation in a gene may have a number of different effects. Some mutations have minimal effects or no effects either on the proteins produced or on the person’s health. Other mutations may lead to abnormal protein production, causing a wide variety of diseases.

The mutated genes that cause hereditary ATTR amyloidosis have important effects on the abnormal so-called ‘variant’ TTR whose production they determine. The differences in structure between the normal and the amyloidogenic variant TTR are usually extremely small but nonetheless they have very important effects on the behaviour of the variant TTR. The variant TTR proteins have an increased tendency to misfold, clump together and form amyloid fibrils. Thus even a very small change in the TTR gene can lead to serious disease.
**Autosomal dominant inheritance**

Complex rules control the inheritance of many characteristics, and of many diseases. Hereditary systemic amyloidosis syndromes like ATTR Thr60Ala are inherited in a fashion known as autosomal dominant. This means that the presence of just one copy of a mutated gene can cause the disease.

When there is simple autosomal dominant inheritance of a condition:

- Each child has a 50% (1 in 2) chance of receiving a mutated copy of the gene from the affected parent.
- Each child has a 50% (1 in 2) chance of receiving a normal copy of the gene from the affected parent.
- Half of the children have a mutated gene and develop the disease. They can then pass the mutated gene and the disease on to half of their children.
- Half of the children have two copies of the normal gene. They are healthy and they cannot pass the disease on to their children.
- Brothers and sisters of people with the disease have a 50% (1 in 2) chance of having the mutated gene and developing disease.
- Men and women have equal chances of receiving the mutated gene and of developing disease.

**Incomplete penetrance**

For many of the diseases that are passed on by autosomal dominant inheritance, all people with a mutation in the gene develop disease. However, for ATTR Thr60Ala amyloidosis, this is not the case. An additional genetic principle called incomplete penetrance operates, making the situation more complicated.

Incomplete penetrance means that:

- Some people who inherit a mutated copy of the gene do not develop any amyloid at all.
- Some people who inherit a mutated copy of the gene develop only a small amount of amyloid and do not suffer from any clinical problems.
- Some patients diagnosed with ATTR Thr60Ala amyloidosis have no family history of the disease.

Information about a particular family is important for evaluation of the likelihood that a young healthy person with a mutation will develop disease.
Appendix 2

Tests that help to diagnose amyloid in the heart

Tests that can help doctors to detect amyloid deposits in the heart include:

**Blood tests: cardiac biomarkers**

When heart muscle is damaged by amyloid deposits, blood tests may detect raised levels of NT-proBNP (N-terminal fragment of brain natriuretic peptide) and high sensitivity troponin. These are known as ‘cardiac biomarkers’. Patients with ATTR amyloid in the heart may have cardiac biomarker levels that are higher than normal.

**ECG**

The ECG test is a safe, rapid, painless method whereby the electrical impulses controlling the heart’s contractions can be detected, measured and represented as a tracing on graph paper.

Some ECG appearances are suggestive of amyloidosis in the heart.

**Echocardiogram**

Echocardiography is an ultrasound test. It is safe and painless and does not involve any exposure to radiation. This test is routinely performed when patients visit the NAC. When amyloidosis in the heart is advanced, it is usually clearly visible on the echocardiogram. However, the findings may be less clear at the early stages of amyloid heart disease.

**Cardiac magnetic resonance (CMR)**

CMR is a method whereby a magnetic field and radio waves are used to obtain detailed pictures of the heart. It is safe and painless, and does not involve any exposure to radiation.

CMR provides very detailed information on the structure of the heart. CMR has been available at the NAC since our new, state of the art CMR unit opened in early 2016. In some patients, echocardiography may not be able to determine whether heart wall thickening is due to amyloidosis or to another cause such as high blood pressure (hypertension). In such patients, CMR imaging can help to distinguish between these different causes of heart wall thickening. When doctors analyse CMR scans, they can often clearly visualise the amyloid deposits within the heart walls, between the heart cells. It is expected that in the future it may be possible to use CMR to accurately measure the quantity of amyloid within the heart wall. Such measurements could then be repeated to follow the build-up of amyloid deposits and their regression with treatment.

**Radionuclide imaging (DPD scan)**

SAP scanning does not provide information about amyloid in the heart. A different radioactive marker that does home in on amyloid in the heart is called 99mTc-DPD, or just DPD. DPD scans are routinely undertaken at the NAC in patients with suspected ATTR amyloid in the heart.

DPD scans are most useful when the deposits are of TTR type, and the amount of DPD taken up by the heart correlates with the amount of ATTR amyloid present. Asymptomatic, ‘early stage’ ATTR amyloid deposits in the heart can sometimes be detected by DPD scan, when other heart tests appear normal.

The DPD scan is obtained in 3D by a method called single photon emission computerised tomography (SPECT), and is combined with a standard CT scan. The doctors can then assess both the structure of the heart and the extent of amyloid deposits.
DPD scanning and SPECT are safe tests. There is only slight exposure to radiation from the tracer and the CT scan.

![Left: DPD scan. Right: fused CT/SPECT scan. Both scans show TTR cardiac amyloidosis. The arrows point at the amyloid deposits in the heart. (Image from Banypersad S et al, J Am Heart Assoc, 2012)](image)

**Biopsy**

**Heart biopsy**

Heart muscle biopsy involves removal of a small sample of heart muscle which is then examined under the microscope to detect amyloid deposits. It is the ‘gold standard’ for diagnosing amyloid deposits in the heart. This means that it is the best available test, against which all other tests are measured. This test is performed by cardiologists or cardiothoracic surgeons.

Patients are usually sedated during the biopsy, which usually takes less than an hour. During the procedure, the doctor numbs a small area of skin in the neck, then inserts a long, narrow tube called a catheter into a blood vessel and threads it through to the heart. X-rays are performed to ensure that the catheter tip is positioned next to the heart wall. Then a grasping device at the end of the catheter is used to remove a tiny sample of heart tissue. The catheter is then withdrawn, bringing the heart tissue with it. The tissue is sent to the laboratory for analysis. The procedure is usually safe and painless, and does not require admission to hospital.

**Nerve biopsy**

Nerve biopsy is a minor procedure for removal of a small sample of nerve, which is then examined under the microscope to detect amyloid deposits. Nerve biopsy is usually taken from one of the following three nerves: the sural nerve (at the ankle), the superficial radial nerve (at the wrist) or the ulnar nerve (at the wrist). These nerves are chosen because any resulting loss of sensation is minor and there is no risk of loss of movement.

The procedure is performed under local anaesthetic, which numbs the area of skin where the biopsy is taken. A small cut is made in the skin and the nerve is exposed, so a small sample can be taken. The wound is then closed using stitches. The procedure is usually safe and painless.

If the biopsy is taken from a nerve in the arm, the patient can usually go home later the same day. If it is taken from the leg, the patient is usually kept in hospital overnight for rest and observation.

**Abdominal fat biopsy**

Frequently, a small piece of fat tissue is taken from the skin of the stomach area (abdominal fat biopsy). The procedure is simple, quick, safe and relatively painless.

It proceeds as follows:

1. A small area of the skin in the stomach area is numbed with local anaesthetic.
2. A needle is inserted into the numb area to take out some fat cells from underneath the skin.
3. The fat cells are preserved and sent to the laboratory for analysis.

**Examination of biopsy tissue**

In the laboratory a special staining technique using a dye called ‘Congo red’ is used to identify amyloid deposits in the tissue sample. Then further stains are used to determine the protein composition of the amyloid fibrils. If amyloid fibrils composed of TTR are present along with the Thr60Ala mutation, then ATTR Thr60Ala amyloidosis is diagnosed.