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# Genetic Testing in Marfan Syndrome

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## Introduction

Marfan syndrome is a dominantly inherited connective tissue disorder affecting mainly eyes, heart and skeleton. The population incidence has been reported as being 1 in 3,300 to 1 in 5,000 live births [1]. Twenty five percent of cases arise as the result of spontaneous mutations and these patients are often more severely affected than the 75% of familial cases [2]. Until the important discovery that the causative gene was located on chromosome 15 and coded for an elastic fibril called fibrillin-1 which provides the core of the elastin fibre in connective tissue [3], the diagnosis was essentially clinical, based on features associated with the syndrome and supportive tests of eye, heart and skeletal involvement. Since the discovery of the gene, many papers have reported mutations, most of them unique, distributed throughout the gene's 65 exons [4].

Mutations can be found in 99% of all clinical cases of Marfan syndrome [5]. These confirm the clinical diagnosis, can be used to screen family members, and provide prenatal screening as well as preimplantation genetic diagnosis.

The type and location of the mutation is also important in predicting the lifelong phenotype, and therefore helping to create a specific management programme [6]. Fibrillin-1 is a cysteine-rich glycoprotein, which is a major structural component of the 10-12 nm calcium-binding extracellular microfibrils [7]. A better understanding of the role of fibrillin-1 is emerging due to the mapping of novel and recurrent causative mutations, and the study of their effect on protein structure and expression.

## Location of Mutation

A region of interest lies between exons 24-32 (neonatal region) [8]. Causative mutations in this region tend to create severe phenotypes and patients usually die in the first years of life of cardiopulmonary failure. A second region between exons 23-29 contains mutations which tend to create phenotypes with no ocular anomalies [9]. A third region is in exons 1-15. Mutations in this region tend to create phenotypes where ocular manifestations are predominant, with very little cardiac involvement [10].

There are 43 calcium-binding epidermal growth factor-like domains in fibrillin-1. The core amino acids are highly conserved. Calcium binds to a highly conserved region in the cbEGF-like domain. Any alteration in the amino acid sequence in this domain results in a failure of calcium binding, and as a consequence the protein may break down in this domain [11]. This type of mutation is most frequently associated with moderate to severe cardiac involvement.

The configuration of the cbEGF-like domain is largely determined through three cysteine-cysteine disulphide bonds. A loss of one of these six cysteines disrupts the normal assembly of the disulphide bonds and therefore destabilises the domain. Also, in some patients the

normal amino acid sequence has been changed to create a new cysteine, which may disrupt the natural assembly of the disulphide bonds [12].

Serious phenotypes are also associated with cases where a transcription of the fibrillin-1 message is prematurely terminated, or part of the FBN-1 gene is deleted. These mutations produce truncated fibrillin-1 polypeptides, or FBN-1 haplo-insufficiency, when one of the genes for FBN-1 is not expressed as a protein, leaving one good gene to do the entire job. One study suggests that patients with haplo-insufficiency respond better to drug treatment [13].

**Mutation Screening Techniques**

The choice of mutation screening technique is largely driven by the success rate, and the cost of the technique. At present, Sanger sequencing technique is very powerful and widely used, but cannot detect large deletions or duplications in the DNA sequence unless it is combined with MLPA technique. This combination has improved the yield of the mutation screening technique to approximately 99% accuracy. However, these techniques do not cover promoter or whole intron regions where variations can occur. Happily, the majority of causative mutations occur within the coding region of the gene.

The latest techniques to be used are called whole genome/exome sequencing, which are part of next generation sequencing (NGS). The difference between them is that whole exome sequencing only screens the coding regions of all the genes in a genome. On the other hand, whole genome sequencing screens the whole genome of a patient including promoter and intron regions. Each variant needs to be subsequently confirmed by the gold standard Sanger sequencing.

Other genes, called modifier genes, could influence the severity of the Marfan syndrome phenotype. Marfan syndrome is clinically variable within a family, and between families. In future, the use of NGS platforms will not only screen the whole FBN-1 gene, but also allow the study of other modifier genes which should help us to understand the genotype-phenotype association in Marfan syndrome patients.

**Overlapping Syndromes**

There are many connective tissue disorders which are similar to the Marfan syndrome phenotype, including Loeys-Dietz Syndrome (LDS), Ehlers-Danlos Syndrome (EDS), Beals Syndrome, MASS Syndrome, Ectopia Lentis (EL), and the growing field of Familial Thoracic Aortic Aneurysm and Dissection (FTAAD) syndromes [14]. If the patient appears classically affected with Marfan syndrome, the FBN-1 gene is usually screened first. If the screening is negative for mutations, a second panel specific for the main presenting feature of the patient, e.g. Ectopia Lentis panel, or FTAAD panel, can be applied economically. It is extremely important to determine the correct underlying gene of any overlapping syndrome, so that the correct diagnosis, prognosis and management plan may be determined. Genetic counselling for the patient, the patient's family and offspring, all depend on mutation identification. This is especially important in the case of a paediatric or adolescent patient where the full phenotype may not yet have developed. If a mutation is not found in the FBN-1 gene for a child suspected of Marfan syndrome, then the parents can be reassured 99% that the patient does not have Marfan syndrome.

**Reproductive Decisions**

<u>Reproductive options in Marfan syndrome</u>

When one parent has Marfan syndrome, each pregnancy and child has a 50% chance of inheriting the mutation. This mutation will either have been inherited (75% of cases), or will

have arisen as a result of a spontaneous change (25%). At present, the technique used for prenatal diagnosis only depends on the fact that a causative mutation has been demonstrated. However, for preimplantation genetic diagnosis, one technique is used for a patient who comes from a family where DNA samples are available from other affected members. A second technique is developed for a patient who has a spontaneous new mutation.

In the rare instance where both parents have Marfan syndrome, there is a 75% chance that the child will be affected, however such a child would be severely affected and the pregnancy often does not proceed to term. Should the child survive, serious complications after birth usually results in neonatal death.

Where one parent is affected and the gene mutation is known, the following options apply:

- prenatal diagnosis (PND)
- preimplantation genetic diagnosis (PGD)
- natural conception
- gamete (egg or sperm) donation
- adoption.

Prenatal diagnosis [15]

This is available during a pregnancy to diagnose whether the foetus has inherited a genetic mutation from the parent. At present, although ultrasound study of the foetal heart and skeletal measurements can raise the suspicion of involvement, it is not possible to diagnose the foetus as being affected in time for termination. The two routinely used procedures for prenatal diagnosis are chorionic villus sampling (CVS) or amniocentesis. Chorionic villus sampling is performed between 10-12 weeks of gestation under ultrasound guidance and can be performed trans-abdominally or trans-cervically. The chorionic villae can be used for DNA extraction and rapid molecular analysis. An additional rapid prenatal test for common chromosomal trisomies is usually offered at the same time to provide added reassurance that the foetus is normal. Results are available for both tests within 5 days. Overall, CVS carries a 1-2% risk of miscarriage. It is important to have full discussion with an obstetric specialist and a genetic counsellor who can explain the techniques, risks and outcomes, and offer support during the decision-making pre- and post-testing.

Amniocentesis

This test is carried out between 14-20 weeks of gestation. With ultrasound guidance, a small quantity (10ml) of amniotic fluid is removed, and foetal cells are isolated. Routinely, levels of alpha foetal protein (AFP) are determined to test for neural tube defect or abdominal wall defect. Molecular analysis of the foetal DNA for Marfan syndrome is determined.

The miscarriage rate with this technique is approximately 0.5-1.0%, slightly lower than the CVS procedure. Support is offered for important decisions regarding continuing or terminating the pregnancy at this stage. If the decision is made to proceed with the pregnancy, preparing for special needs, medication and support that the baby may need would be encouraged, to help parents plan for the birth.

Non-invasive genetic testing (NIPT/NIPD)

DNA cell fragments are always present in maternal blood, and during pregnancy approximately 10% of these fragments are from the foetus, arising as a result of placental cell death. This fraction is known as cell free foetal DNA (CFFDNA), compared to the maternal fraction, CFMDNA. At the time of writing, NIPT is commercially available for

aneuploidy (chromosomal number) screening in the private sector and is being assessed for possible routine antenatal care across the NHS. At this time, testing is not currently available routinely for Marfan syndrome, since there are no common mutations in the FBN-1 gene. However, if the FBN-1 family mutation is known, it is possible to develop a unique prenatal test for NIPD. The work up time is approximately 8 weeks, so it is essential that the test is prepared prior to pregnancy. Once the test is available, the turnaround time for a diagnostic result at 10 weeks of pregnancy is 5 days. One of the major advantages of NIPT/NIPD is the reduction of miscarriage risk. Another advantage is that a definitive diagnosis can be made at an earlier stage in the pregnancy. This technique may be available in the future. The main limitation is that it is not possible to distinguish between DNA fragments in multiple pregnancies. Follow-up screening by CVS or amniocentesis would be recommended. In all instances, the legal limit on termination of affected pregnancies would need to be observed.

Preimplantation genetic diagnosis (PGD)

For some couples, termination of an affected pregnancy is not an option due to religious and cultural beliefs. An alternative to prenatal diagnosis can be considered, by utilising preimplantation genetic diagnosis [16]. PGD is performed by combining in vitro fertilisation (IVF) treatment with genetic analysis of single cells removed from embryos to test for the presence of the specific genetic alteration prior to embryo transfer and implantation. The oocytes (eggs) are retrieved following ovarian stimulation and are fertilised in the laboratory using either IVF or ICSI (intracellular sperm injection). Single cells are removed from each embryo on Day 3 of embryo development, or multiple cells removed at Day 5 or 6. DNA from the biopsied cells is processed and analysed. Embryos diagnosed as unaffected are transferred into the uterus. Embryos diagnosed as affected are, with the couple's consent, humanely discarded.

All PGD tests are unique and specifically created for couples, a process which is time-consuming and labour intensive. The technique of karyomapping is now becoming increasingly used for single gene disorders [17]. A blood sample is taken from the prospective parents, one of whom has an FBN-1 mutation, and another relative who may or may not be affected by the condition. This may be the couple's child, or a first degree relative of the affected parent. This relative is referred to as the 'reference'. If the mutation is de-novo in the parent, the reference can either be a parent or sibling of the affected individual. If there are no relatives available, embryos produced in the PGD cycle can be used as the reference. Using this method it is possible to detect the chromosome which carries the altered gene. If the embryo has inherited the chromosome carrying the defective gene, that embryo would not be used for embryo transfer. If the embryo is not carrying the mutation, it is predicted to be free of the disorder and is suitable for transfer.

PGD can be stressful for some couples as there are many stages in the process and there are no guarantees that the treatment will result in pregnancy. In Marfan syndrome, couples usually have no underlying infertility issues, hence the success rates are slightly higher than for couples suffering from infertility.

Natural conception

Spontaneous pregnancy is an option available to all fertile couples. The decision whether to check the pregnancy for Marfan syndrome prior to birth depends on the couple's cultural and religious beliefs. If there are strong objections to terminating an affected pregnancy, discussions with healthcare professionals would be encouraged to prepare for the outcome of a child with Marfan syndrome. Mutation screening can be offered in the postnatal period, using cord blood or a separately obtained sample. Routine antenatal care and ultrasonography can be performed to check the development of the foetus and foetal heart in the first and second trimesters. It is not possible to predict the severity of the condition in an

affected pregnancy, due to variability of gene expression, although the parent's condition and that of other affected family members will give some indication.

Gamete (egg or sperm) donation

The donor conception network in the UK has a wealth of information for prospective recipients and is recommended for any patient considering this option [18]. A major change in the last few years is the introduction of non-anonymity for donors in the UK, such that a donor-conceived child at the age of 18 can contact their genetic parent. The register is maintained by the HFEA (Human Fertilisation and Embryology Authority) [19]. A couple may choose to use a known or anonymous egg or sperm donor. Gamete donors are routinely screened and offered appropriate genetic testing and screening.

Adoption

Adoption is a way of providing a permanent home and family to a child who is unable to be raised by their birth family. If one parent has a genetic condition, the adoption agency is responsible for making an assessment in the best interests of the child. Therefore the diagnosis, prognosis, and effect on the long-term health of the parents, should be factored into the decision. If the child to be adopted has Marfan syndrome, the adoptive parents must be given full and accurate information about the effects of the condition and the prognosis for the child in order to ensure that adequate provision is made for the child [20,21].

## Conclusions

As a result of intense international collaborative clinical and molecular genetic research in the past 23 years since the cause of Marfan syndrome was identified, an internationally available gene map has been drawn up containing several thousand mutations, most of them unique [12]. This has provided the basis for diagnosis and genotype-phenotype correlations, which now permit accurate confirmation of clinical diagnosis, or point the clinician to screening for an overlapping disorder. Long term prognosis and management programmes can now be based on the identified mutation type and location within the gene. Rapid reliable laboratory techniques are now widely available to identify mutations, and the Marfan gene is being included in panels of genes used to screen patients with thoracic aortic aneurysms, dislocated lenses, and growth disorders, which should lead to increased diagnostic accuracy in patients with these clinical problems.

For affected persons, the availability of mutation testing has been of major importance in aiding informed reproductive decisions. Assisted reproduction offers safe reliable help in conceiving an unaffected child, especially useful in severely affected families with visual loss or early cardiac death. Reproductive options include natural conception, gamete donation, or adoption, as well as prenatal diagnosis, and preimplantation genetic diagnosis (PGD), and in our experience the acceptance of these latter new techniques is high in affected couples.

Families and affected couples should be encouraged to seek full information through referral to their regional genetic centre, and a whole team of supportive health personnel will be available at all stages to assist in informed decisions, and guarantee best use of genetic testing as a basis for personalised medical management programmes.

## Short introduction

Genetic testing, now widely available throughout the UK for Marfan syndrome, is aiding rapid diagnosis as a basis for management programmes of eye, heart and skeletal disease. The affected patient's mutation can also be used as a basis for prenatal or postnatal diagnosis of each offspring which bears a 50% chance of being affected. Preimplantation genetic

diagnosis can ensure an unaffected pregnancy, and is now widely accepted as the technique of choice.

## Conflict of interest

Drs Child, Aragon-Martin and Sage have no conflicting interests to report.

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**Key Points**

1. Marfan syndrome affects 1 in 3,300 population worldwide.

2. The dominant inheritance is familial in 75% of cases. 25% of cases are sporadic.

3. In 99% of classically affected patients with 2/3 major systems affected (eyes, heart, skeleton), a causative mutation is demonstrated.

4. An international map of the causative gene fibrillin-1 contained several thousand mutations, most of which are unique.

5. Close family members can be screened by the Regional Genetics Centre who can also refer for prenatal or postnatal pregnancy screen (50% genetic risk for each pregnancy of affected patient).

6. Prenatal mutation testing by CVB is offered at 11 weeks gestation; by amniocentesis is at 14 weeks.

7. Preimplantation genetic diagnosis is available through NHS or privately with 40% chance of success on first attempt.

8. Further information is available through the Marfan Trust at www.marfantrust.org.