British Heart Foundation

NHS
UK Genetic Testing Network

The new cardiac genetic testing panels: implications for the clinical cardiologist

Meeting report
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UK Genetic Testing Network

Authors

Ms Louise Cameron

Dr Shehla Mohammed

Ms Jane Deller

Dr Mark Kroese

Acknowledgements

Dr Paul Clift

Professor Perry Elliott

Professor Clifford Garratt

Professor Bernard Keavney

Dr Kay Metcalfe

Professor Jeremy Pearson

Professor Nilesh Samani

Dr Fiona Stewart MBE

Professor Hugh Watkins

Dr Maggie Williams

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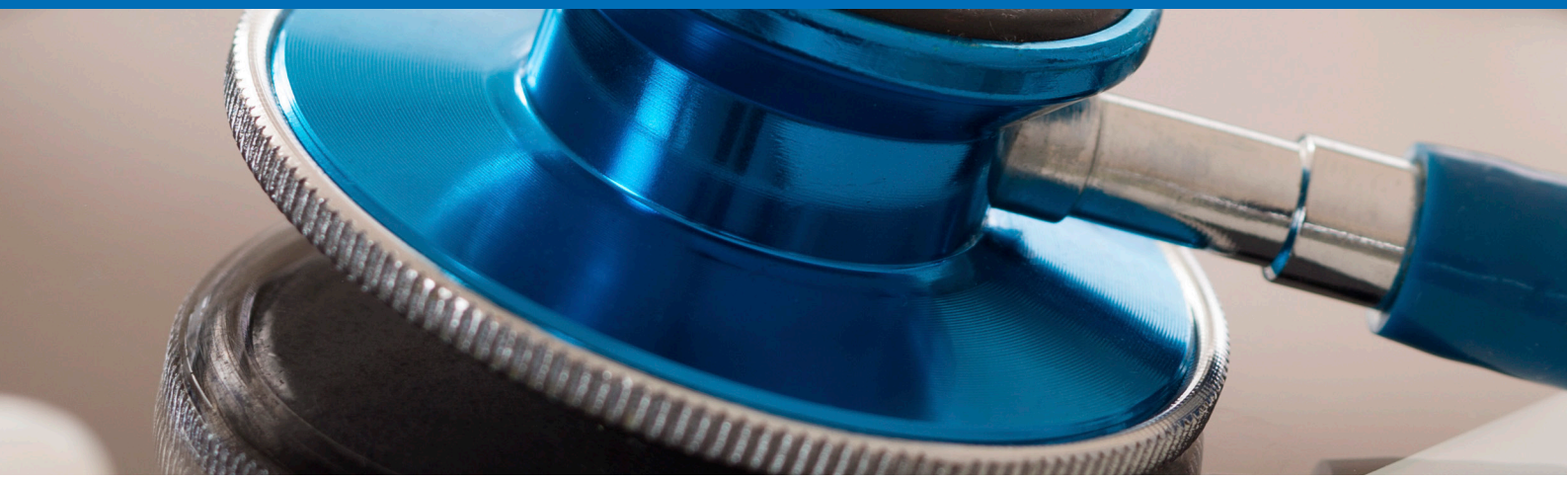
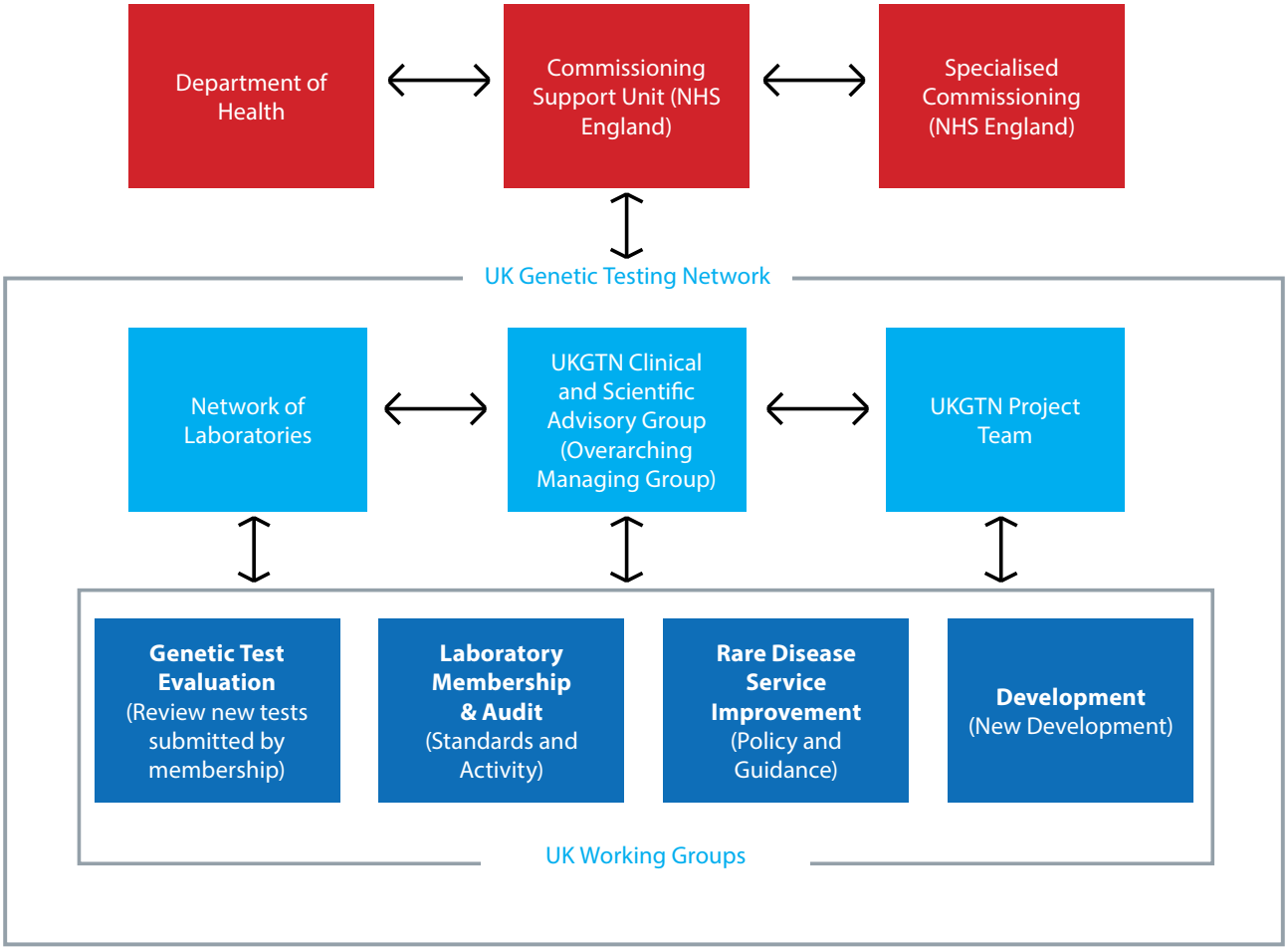


On 8th June 2015, the UK Genetic Testing Network (UKGTN) co-hosted, with the British Heart Foundation (BHF), two sessions at the British Cardiovascular Society (BCS) annual conference, which was held in Manchester. The overall objective of these sessions was to raise awareness of the latest developments in genetic testing for cardiac conditions. Attendees included NHS consultant cardiologists, specialist registrars (trainees) in cardiology and other interested clinicians from across the UK. This report summarises the details of the presentations and the discussions that took place.

UK Genetic Testing Network

The UKGTN is a national advisory organisation for NHS genetic testing services. It was set up by the Department of Health in 2002 to promote equity of access to gene testing within the NHS. It is a collaborative of clinicians, scientists, patient representatives and commissioners and has a membership of laboratories. Over 60 colleagues from the UK clinical genetics community provide advice to four working groups in the delivery of the annual work programme. The member laboratories are in the main, but not exclusively, associated with NHS Regional Genetic Centres within NHS tertiary Trusts. The laboratories apply to be members of UKGTN and are accepted providing they meet the required quality criteria. The UKGTN is supported by the project team, advisors and chairs of the working groups. The accountability is through the UKGTN Clinical and Scientific Advisory Group that has a wide representation from the member nations, professional bodies, the Department of Health and patient groups. The work of the UKGTN influences policy development, provides advice to healthcare commissioners, assures quality of laboratories and the network services they provide and evaluates and recommends new genetic tests for NHS service.

Figure 1 UKGTN organisation



British Heart Foundation

The British Heart Foundation is the leading funder of university-led cardiovascular research in the UK with an annual research spend of around £100 million. Approximately, a further £30 million is spent annually on its other charitable objectives, including support and information for the public and patients, together with policy and advocacy work.

The BHF's research aims are to:

- Increase investment in world-class research to combat cardiovascular disease
- Ensure that research funded by the BHF and others translates into better prevention, diagnosis and treatment outcomes

The BHF's three further strands of work are grouped under the headings of *Prevention*, *Survival* and *Support*:

- Prevention focuses on empowering people to make healthy choices around physical inactivity, smoking, high blood pressure, elevated cholesterol and obesity to reduce their risk of cardiovascular disease
- Survival is committed to creating a 'Nation of Lifesavers'; leading the fight to ensure more people survive a heart attack or cardiac arrest through cardiopulmonary resuscitation (CPR) training and defibrillator awareness
- The Support programme works to ensure that everyone in the UK with cardiovascular disease has access to high quality, integrated health and social care services, and to empower people living with cardiac conditions and cardiovascular disease to manage their condition through access to high quality information, support and guidance

The BHF has a long history of involvement in research and development of genetic testing for cardiovascular conditions.

In the 1990s two promising young researchers, Drs Hugh Watkins and Bill McKenna (later to be BHF Professors), funded by the BHF, were amongst the first to identify genes underlying hypertrophic cardiomyopathy (HCM). The BHF has subsequently consistently funded research to uncover the genetic causes of the various forms of cardiomyopathy and the 'channelopathies' that can lead to sudden cardiac arrest, to understand how they cause the conditions and potential avenues for treatment-ultimately including gene therapy. Current investment in BHF-funded research in this field is more than £10 million. As the presentations in this report summarise, this basic research has now produced sufficient evidence to enable informative genetic testing for HCM and for this to be provided as an NHS service in the UK.

Even earlier, in the 1980s, the BHF began to fund Dr (now BHF Professor) Steve Humphries in his search to identify the genetic causes of familial hyperlipidemia (FH). Continuous BHF funding for Professor Humphries and colleagues, together with his strong advocacy, led to greater recognition of the frequency of FH (perhaps 1 in 250 of the population, of whom many are still undetected) and the production in 2008 of national guidelines for genetic testing. With BHF support, the first cascade testing service for FH was set up in Wales and is currently being extended to the rest of the UK.

The BHF is proud to be strongly associated with both these areas of genetic testing, which together represent excellent examples of the need for long-term investment in basic and translational research to bring scientific discoveries to the point where they benefit patients and the public.

UKGTN genetic test evaluation process

Overview

A presentation on the UKGTN genetic test evaluation process was provided by the UKGTN Clinical Advisor, Dr Shehla Mohammed.

The genetic test evaluation process (previously referred to as the Gene Dossier process) was developed by the UKGTN in 2003 as a tool to evaluate whether a proposed laboratory genetic test for a specific genetic disease is to be recommended for inclusion on the NHS Directory of Genetic Disorders/Genes for Diagnostic Testing (previously NHS Directory for Genetic Testing). Once a test is on the Directory it is recommended to be considered for funding under local commissioning arrangements. The Directory lists disease and gene combinations for which tests are available and NGS panel tests that have been agreed as appropriate for clinical use, from member laboratories. Information about the testing services provided and the laboratories providing them are available from the online database on the UKGTN website. The process ensures that the decision regarding the recommendation of a test is explicit, transparent and based on evidence. The genetic test evaluation documents and a description of the process can be found at www.ukgtn.nhs.uk/resources/genetic-test-evaluation-process.

The genetic test evaluation form (gene dossier)

The process requires laboratories to submit a form called a 'gene dossier' for evaluation by the Genetic Test Evaluation Working Group (GTEWG). The membership of this group includes professionals from Clinical Genetics, clinical laboratory genetics, Public Health, commissioning and patient groups. The gene dossier provides a standardised format for the evaluation of the key information about a genetic test including analytical validity, clinical validity and clinical utility. Laboratories submit a shortened version of the form, called an additional provider form, to request listing of a test under their laboratory on the UKGTN website where the test is already on the NHS Directory of Genetic Disorders/Genes or on the UKGTN website.

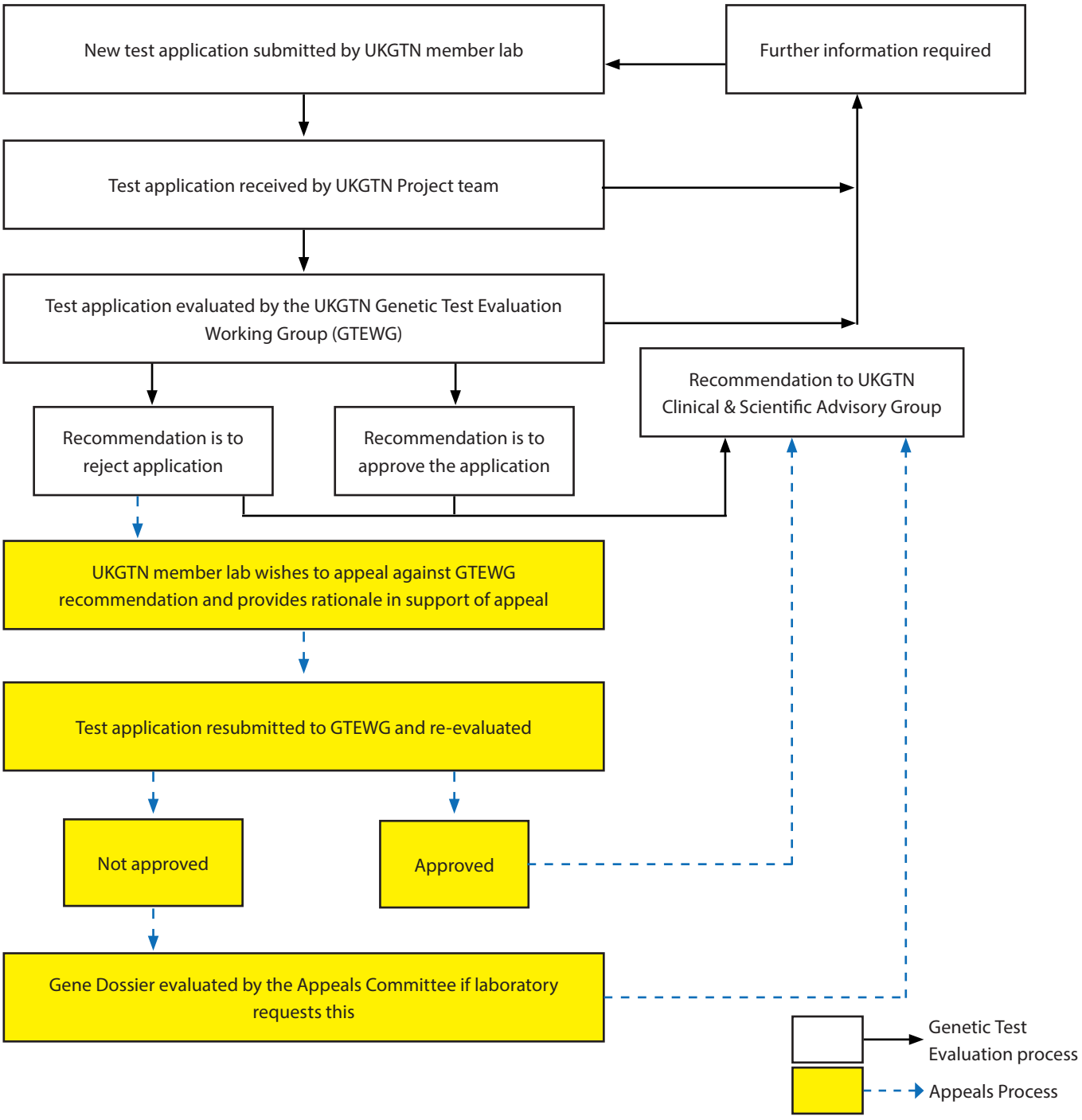
Testing criteria

Every application for a new test that is submitted has to include testing criteria. The UKGTN developed the concept of testing criteria as part of the new test application process. Testing criteria define the appropriateness of a genetic test referral, and it is intended that the test is only carried out in accordance with the criteria as set out in the gene dossier and approved by the UKGTN Clinical and Scientific Advisory Group. Testing criteria should include only those data that are specified within the gene dossier, and should not be confused with any other information that a provider laboratory may wish to have for research

or any other reasons. The additional benefit of these criteria is that they can inform clinicians' decisions about which investigations are suitable for their patients.

In addition to developing testing criteria as part of the test evaluation process, the GTEWG also develops testing criteria for tests that have been on the NHS Directory of Genetic Disorders/Genes prior to the introduction of testing criteria. The UKGTN project team organises conferences/workshops on specific disorders for scientists and clinicians in order to develop consensus testing criteria. This promotes a consistent approach to genetic test provision for these conditions throughout the UK. The UKGTN has used this method to develop testing criteria for Cystic Fibrosis, Fragile X, Marfan syndrome and familial breast and ovarian cancer.

Figure 2 UKGTN genetic test evaluation process



Tests that the UKGTN will evaluate

The UKGTN will evaluate any new genetic test that a UKGTN laboratory member wishes to provide and have listed on the NHS Directory of Genetic Disorders/Genes for Diagnostic Testing. For the UKGTN genetic test evaluation purposes, prior to April 2013, a genetic test was defined as any test for NHS service provision by a UKGTN member laboratory which required funding by specialised commissioning arrangements, supporting provision of clinical genetics services as defined in the national definition set for medical genetics services. Since April 2013, the definition of a genetic test for UKGTN evaluation has been expanded to include tests for any prescribed specialised service. There has been a steady increase in applications over the years followed by a recent decline due in part to an increasing number of large single NGS panel test applications covering a number of genes and associated disorders.

The evaluation

It is recommended that new test applications are completed by the UKGTN laboratories in collaboration with clinical colleagues with relevant specialist expertise. The GTEWG undertakes the evaluation of the proposed new test.

The evaluation is based on the ACCE (Analytical validity, Clinical validity, Clinical utility & Ethical, Legal and Social) framework[1] and takes into account the following:

1. The seriousness of the condition
2. The prevalence of the condition
3. The purpose of the test- diagnosis, treatment, prognosis and management, presymptomatic testing, risk assessment
4. The technical details of the test
5. The context in which the test is to be used- defined population groups
6. The characteristics of the test- the clinical sensitivity, specificity and predictive value
7. The clinical utility of the test- how it adds to patient management and the availability of alternative diagnostic procedures
8. Ethical, legal and social considerations
9. The price of the test

Test applications are also assessed for the following healthcare outcomes:

- Alerts to significant clinical co-morbidities
- Reduces mortality/saves lives
- Avoids irreversible harm
- Avoids diagnostic invasive procedures/tests (some of which may be invasive) and/or multiple hospital appointments
- Avoids incorrect management (*e.g.* medication or treatment) that could be harmful
- Confirms targeted therapy/management
- Earlier diagnosis allowing commencement of treatment earlier with associated improved prognosis
- Enables access to educational/social support
- At risk family members that test negative for a familial mutation can be discharged from follow up
- At risk family members that test positive for a familial mutation have appropriate follow up

Frequency of evaluation cycles

Prior to 2014 the process was carried out annually (over a nine month period from submission to recommendations being made) with recommendations being made to the September CSAG meeting. From 2014 the process became biannual with recommendations being made to both the March and September CSAG meetings. The two deadlines for gene dossier submissions to UKGTN are 31st January (for recommendations made to the September CSAG within the same year) and 31st July (for recommendations made to the March CSAG in the following year).

Commissioning

The results of the evaluation are presented to the UKGTN Clinical and Scientific Advisory Group (previously UKGTN Steering Group) for endorsement. Following this endorsement the recommendations are reported to NHS England and equivalent organisations in Wales, Scotland and Northern Ireland. Each devolved nation follows its own process to consider adoption of the tests. UKGTN approved tests are added to the NHS Directory of Genetic Disorders/ Genes for Diagnostic Testing and the UKGTN online database. Both of these resources are publically available from the UKGTN website (www.ukgtn.nhs.uk).

[1]Haddow J, Palomaki G. ACCE: A Model Process for Evaluating Data on Emerging Genetic Tests. Human Genome Epidemiology. Khoury M, Little J, Burke W, eds. Oxford: Oxford University Press, 2004; 217-233

Monitoring the introduction of UKGTN recommended new tests

The UKGTN monitors the activity and funding required for new tests that have been approved two years after they have been recommended for national NHS service. This provides a comparison of the real activity and costs against those predicted in the application forms. This is shared with the Medical Genetics Clinical Reference Group and any large differences identified as part of this national audit are investigated by UKGTN to establish the reasons for the disparity.

The UKGTN first evaluated panel tests that used Next Generation Sequencing Technology (NGS) in 2011 and between 2011 to March 2015 approved and recommended 55 NGS panel tests of which there were 106 sub panels. A sub panel is defined as a test for a number of disorders that present with similar clinical phenotypes. A test using Whole Genome Sequencing (WGS) was also recommended in this period.

Further information about the number of test applications that UKGTN has evaluated since 2004 is shown in Figure 3. More detailed information about the number of evaluations recommended for service from April 2015 is shown in Table 1.

Figure 3 UKGTN evaluation of new genetic tests 2004-2014

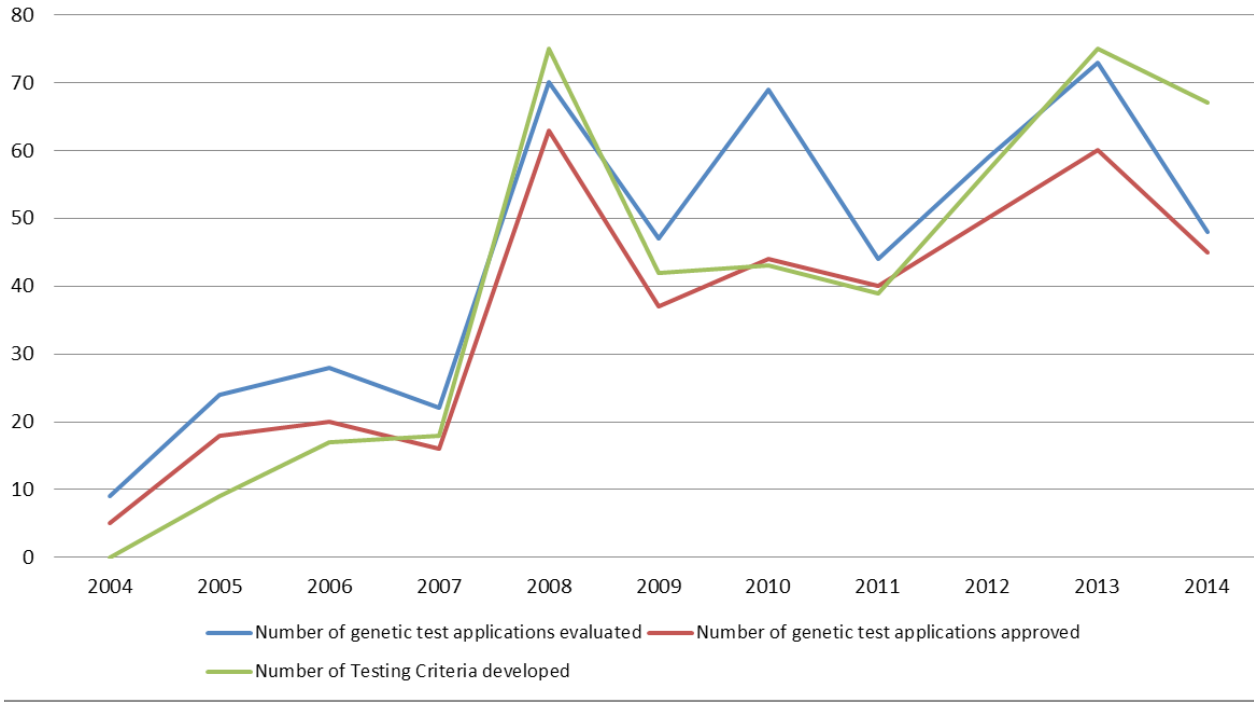


Table 1: New genetic test recommendations for NHS service from April 2015

Genetic test applications evaluated in 2014	48
New tests recommended and approved	45
Genetic test applications with savings across diagnostic care pathways	17
Genetic test applications with fewer than 50 index cases per annum	29
New panel tests (NGS)	23
New NIPD* tests	2

*Non-invasive prenatal diagnosis

Presentation summaries

1. The emergence of new genetic tests for cardiac disease; what the cardiologist needs to know

Professor Clifford Garratt, University of Manchester

Introduction

The move from Sanger sequencing to next generation sequencing (NGS) methods has facilitated a move away from single gene sequencing to wider interrogation of the genome. NGS relies on fragmentation of genomic DNA to generate large amounts of sequence reads which are aligned to a reference genome to identify variants, with the use of bioinformatic tools. The principal advantages of NGS are its capacity, its efficiency in covering a much larger proportion of the genome, and its relatively low cost. Greater amounts of information can be derived, however not all of it is useful in a clinical context. Sanger sequencing in contrast is relatively time consuming and expensive, but owing to its high accuracy, it remains the gold standard test for definitive confirmation of single gene variants, even in the era of NGS.

Genetic testing panels in cardiovascular disease

Genetic testing panels for cardiovascular disease incorporating NGS methods may be highly targeted, for example for Long QT (LQT) syndrome, which tests for around 5-15 genes. A larger panel, for example, for cardiomyopathy, may test for around 20 genes. A much wider approach would involve sequencing the whole exome or whole genome. The advantage of panel testing is that it supports interrogation of several genes in conditions which have a polygenic aetiology and is useful when the phenotype does not point towards a particular gene as, for example, with dilated cardiomyopathy (DCM). The two main drawbacks of wider testing are the generation of variants of uncertain significance (VUS) and incidental findings (IFs).

Variants of uncertain significance (VUS)

In the context of cardiovascular disease, a small number (around 3-4%) of normal individuals have a unique variant that alters the amino acid sequence of one of the sodium or potassium channel proteins which might be interpreted as a 'positive' result in patients with LQT syndrome.

Incidental findings (IFs)

Exome screening will identify a substantial number (around 200) novel protein-altering single nucleotide variants in each individual, and this may include genes relevant to other conditions unrelated to the reason for testing, for examples genes associated with cancer or dementia risk.

Interpretation

Interpreting the information derived from NGS is therefore critical to establish whether the gene mutation is disease-causing in the individual patient. This probabilistic process is heavily dependent on the pre-test probability of disease and, as with all genetic tests, high quality phenotyping and clinical assessment is of critical importance, but particularly so when testing for a wider number of variants.

Case study

A 19 year old asymptomatic woman was assessed immediately following the death of her sister aged 17 years, following a series of 'faints.' A post-mortem diagnosis of arrhythmogenic right ventricular cardiomyopathy (ARVC) was made in her sister. Examination of the patient showed normal echocardiogram and cardiac magnetic resonance (MR) scan, but an ECG showed anterior T wave inversion. At the time the cardiologist concluded that the patient probably had ARVC, but was at low risk in light of the accompanying clinical picture.

Ten years later the patient re-contacted the clinical team, following the birth of her first child, wishing to revisit the issue of familial cardiac disease. The patient was referred to the clinical genetics service and the familial arrhythmia clinic for assessment. She was referred for a further cardiac assessment, and the ECG did show anterior T wave changes but also a prolonged QT interval, the presentation being typical of Long QT 2 syndrome (LQT2 syndrome). This finding was also seen on the mother's ECG. Genetic testing for the appropriate phenotype (LQT syndrome) revealed a mutation in the cardiac potassium channel *KCNH2* gene, with published evidence of this being a causative gene for LQT2 syndrome.

This led the team to re-evaluate the ARVC diagnosis in the proband. The typical phenotypic features of LQT2 syndrome are syncope or cardiac arrest associated with sudden auditory stimuli. Details in the proband's case notes were consistent with this, particularly the past history of syncope in relation to auditory stimuli at night. An ECG was found in the proband's GP records which suggested LQT syndrome. It is possible that the proband had both ARVC and LQT syndrome but much more likely that LQT syndrome was the cause of death.

Role of genetic testing in diagnostic process

This case illustrates that, whilst genetic testing can be usefully employed in the diagnostic process, it is not a good alternative to making a clinical diagnosis, and careful consideration should be given before proceeding to genetic testing. The Heart Rhythm Society and the European Heart Rhythm Association consensus statement about genetic testing state that:

"Genetic testing for LQT syndrome should not be performed solely on the basis of a past history of syncope, as part of pre-sports participation or as a universal screening protocol. Nor is it recommended for diagnosis of hypertrophic cardiomyopathy (HCM) patients with non-diagnostic clinical features."

Manchester cardiac genetic panel

Preliminary results for the Manchester cardiac panel were presented. The panel tests for genes associated with catecholaminergic polymorphic ventricular tachycardia (CPVT), Brugada syndrome, LQT syndrome, hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), ARVC, aortic dilation, arrhythmia/cardiac arrest, cardiomyopathies and a set of genes for molecular autopsy.

For HCM, 151 patients were tested and 65 variants detected (43%), where only 35 would previously have been detected. For both HCM and DCM combined there was a 74% pickup rate using the Manchester panel, as compared to 47% with previous testing, with 10 additional variants detected.

For arrhythmia, the results were slightly less marked: the panel pickup rate was 57% versus 29% with previous testing, with 12 additional variants detected.

For Brugada syndrome, LQT syndrome and CPVT genes the results were less marked still: with equivalent pickup rates for the former two and a 6% uplift for the latter when compared to previous testing.

Conclusions

NGS is an efficient and relatively inexpensive method for examining a very large number of genes. If genetic testing is indicated in the proband, targeted gene panel testing in conjunction with high quality clinical evaluation would be recommended, coupled with thorough pre-test genetic counselling and expert interpretation of genetic results.

2. Sudden cardiac death syndromes 62 gene panel

Dr Kay Metcalfe, University of Manchester

Benefits of genetic testing in cardiovascular disease

Genetic testing in cardiovascular medicine is mostly conducted in individuals who have a clear clinical diagnosis, and genetic testing may be helpful to maximise the impact of targeted therapies within a therapeutic window, to prevent complications in the patient, and to benefit the family in terms of cascade screening and reproductive risk estimation. Genetic testing may also direct appropriate clinical investigations and avoid unnecessary procedures.

Manchester cardiac genetic testing panel

The Manchester cardiac genetic testing panel can interrogate genes associated with specific cardiovascular conditions, for example cardiomyopathies or arrhythmias. The molecular autopsy panel examines genes associated with a number of conditions which may be the cause of sudden cardiac death. This does not include conditions which would be clearly apparent at post-mortem and therefore genes associated with aortic aneurysm are not examined. The price of testing (for NHS patients) for initial analysis of each set of genes ranges from £700 to £1100. Subsequent requests for other gene sets is slightly lower as this represents analysis costs only and not re-processing and re-sequencing costs.

UKGTN testing criteria* for conditions associated with sudden cardiac death

The UKGTN testing criteria outline the clinical features for a number of conditions which may result in sudden cardiac death and for which panel testing may be carried out, for example LQT syndrome, CPVT, and Brugada syndrome. Family screening would not involve panel testing, but rather targeted Sanger sequencing of the gene identified. The testing criteria for the arrhythmias differ slightly as panel testing for arrhythmias may occur following cardiac arrest without an underlying primary cardiac diagnosis.

Variants: pathogenicity scoring

In assessing the pathogenicity of an observed variant, the interpretation takes into account several factors including: if the variant has been seen before, if it is noted in large databases of normal populations, if it is a conserved residue in the DNA down evolution, and how it impacts on the amino acid sequence.

Variants are classified into five types:

1. Clearly not pathogenic - common polymorphism
2. Unlikely to be pathogenic - diagnosis not confirmed molecularly
3. Uncertain pathogenicity - does not confirm or exclude diagnosis
4. Likely pathogenic - consistent with the diagnosis
5. Definitely pathogenic - this result confirms the diagnosis

*see Appendix 1 for UKGTN testing criteria

The UKGTN testing criteria recommend testing in cases of sudden cardiac death under 40 years of age in the presence of normal morphology, with or without a family history.

Class 3 variants with uncertain pathogenicity are the most problematic in terms of patient care, as careful consideration must be given to whether it is beneficial or harmful to feed back this information to the patient.

Results of Manchester cardiac genetic testing panels

Results demonstrate that 24 variants were picked up in 42 patients using this panel to test for arrhythmia and cardiac arrest, but only 15 were class 4 or 5 variants. Very little uplift in variant detection was noted for LQT syndrome, Brugada syndrome and CPVT, ranging from 1-20 variants detected with between 0 and 11 being class 4 or 5 variants.

Sudden cardiac death

Sudden cardiac death is responsible for 100,000 deaths each year in the UK, with the majority of cases secondary to coronary heart disease. However, most deaths in people under 30 years of age are as a result of inherited cardiomyopathies or arrhythmia. Sudden cardiac death accounts for 10% of deaths in people aged between 1 and 22 years, and in around 20% of cases under the age of 35 no identifiable cause can be found at autopsy. Post-mortem examination may assign a structural cause to the sudden cardiac death. However there may be a small subset of patients in whom a structural cause is not apparent. A substantial proportion of these may be diagnosed with an arrhythmia through the use of the molecular autopsy panel.

Finding a cause

Establishing a diagnosis can explain why a person has died and provide information for relatives, and screening to other family members. Independently, cardiac screening in relatives can offer a diagnosis in around 50% of families. A detailed history from the deceased and family history, along with expert post-mortem examination, are critical to finding a cause and can now be bolstered by the use of a molecular autopsy genetic testing panel. DNA is now routinely taken following a sudden cardiac death. However it is important to acknowledge that a negative result from genetic testing does not rule out a genetic cause, and there is also the possibility of finding variants of unknown significance (VUS).

Genetic testing following sudden cardiac death: published studies

Results were presented from published studies using genetic testing following sudden cardiac death[2,3,4,5,6,7]. Most of the studies had focused on the LQT syndrome genes and exons of *RYR2* for CPVT and used Sanger sequencing. One study using whole exome sequencing by Bagnall *et al.*[6] described 50 cases of sudden unexplained death in patients aged between 1 and 40 years, 48% of whom died in their sleep. Exome sequencing was carried out on a subset of 28 patients and found three rare variants in LQT syndrome genes and six rare variants in 25 genes associated with arrhythmia and cardiomyopathy. Yields in the studies were heavily dependent on methodology, selection of patients and quality of DNA samples and ranged from around 15 to 30%, but some of the reported variants were putative pathogenic. One of the difficulties of testing in this area is that the phenotypic information is limited to the occurrence of a sudden cardiac death.

Challenges of exome/genome sequencing approaches in sudden cardiac death syndrome

Universal challenges in NGS approaches include the generation of large volumes of data to be interpreted, the occurrence of incidental findings, consent to these being reported and the multigenic aetiology. Specific challenges of NGS approaches in cardiovascular disease include the difficulty of determining pathogenicity in the absence of

[2] Skinner *et al.* 2011 Heart Rhythm 2011; (8)3: 412-9
[3] Tester *et al.* 2012 Mayo Clin Proc 2012; 87(6): 524-39
[4] Doolan *et al.* 2008 Int J Cardiol 2008; 127(1): 138-41
[5] Chugh *et al.* 2004 J Am Coll Cardiol 2004; 43(9): 1625-9
[6] Bagnall *et al.* 2014 Heart Rhythm 2014; 11(4): 655-62
[7] Winkel *et al.* 2012 J Cardiovasc Electrophysiol 2012; 23(10): 1092-8

a detailed phenotype, as the clinical indication for testing is death of the patient, and the difficulties associated with consent surrounding post-mortem samples.

Recommended approach to testing

The Heart Rhythm Society (HRS) and European Heart Rhythm Association (EHRA) expert consensus statement recommends that tissue or blood samples are taken and stored in cases of sudden infant and sudden unexplained cardiac death. If the autopsy is negative, consideration should be given to targeted gene testing on the proband and relatives where appropriate, particularly if there is any clinical information which would indicate LQT or CPVT was present.

The UKGTN testing criteria recommend testing in cases of sudden cardiac death under 40 years of age in the presence of normal morphology, with or without a family history.

Manchester molecular autopsy panel

Results of testing with the Manchester molecular autopsy gene panel in 29 patients were presented and showed 22 variants identified in 15 patients. All identified variants were given a pathogenicity score of either 3 or 4 so represented either VUS or variants likely to be pathogenic. Most of the mutations in the *RYR2* gene (associated with CPVT) were assumed to be pathogenic. The value of the wider molecular autopsy panel was apparent as some of the mutations identified would not have been picked up from testing with only the LQT syndrome and CPVT panels.

Case study

A case study was described with a sudden unexplained death in a baby aged 13 months. Genetic testing revealed the child had a variant in the *SCN3B* gene (linked to the *SCN5A* gene) which was maternally inherited. The finding was initially reported as a VUS by the laboratory, although mutations in this gene have been reported in cases of Brugada syndrome. On the basis of this, the coroner's report suggested that the cause of death was most likely to have been arrhythmia. The parents have gone on to have another child who does not carry the variant, and has had normal results from cardiac screening. Functional studies are underway to establish if this variant is pathogenic.

Panel testing in a clinical setting

The utility of panel testing was emphasised with a pedigree showing several cases of sudden death across three generations of a family, before a referral to clinical genetics which led to a variant causing CPVT being identified within the family.

Summary

Genetic testing may be helpful in the context of sudden cardiac death but the process is probabilistic and constitutes one element of a comprehensive clinical evaluation. Larger gene panels allow testing for rarer causes but there is a greater likelihood of returning VUS. Generally, genetic testing is carried out in the context of clinical diagnosis, but it may also be useful in cases of cardiac arrest and sudden death where a clinical diagnosis is not available.

3. Familial thoracic aortic aneurysm syndromes and Marfan syndrome

Dr Paul Clift, Queen Elizabeth Hospital, Birmingham

Introduction

Familial thoracic aortic aneurysm syndromes (FTAA) include Marfan syndrome and are known to have a genetic basis, with panel testing proving a useful aid to diagnosis. Index cases may present with aortic dissection or unexpected findings on routine investigation. Individuals may also present with a family history either with evidence of aortic dilatation themselves or through a screening process which identifies relatives of patients who have died from an aortic dissection.

Clinical management

Historically, initial management has involved surgery and anti-hypertensive therapy, with referral to clinical genetics if Marfan syndrome was considered likely. Patients would then receive surgical follow-up and referral to the local cardiology service, with no further testing.

The identification of the Fibrillin 1 (*FBN1*) gene mutation along with mutations in the TGF-β receptor (in Loeys-Dietz syndrome, a very aggressive aneurysm syndrome) catalysed a changing approach to clinical management of these conditions. A number of other syndromic conditions have subsequently been identified including Ehlers-Danlos syndrome and arterial tortuosity syndrome. In addition, a demonstrable genotype has been identified in a substantial proportion of patients who do not have defined phenotypic features.

Marfan syndrome

The cardinal features of Marfan syndrome are aortic root aneurysm and ectopia lentis. Other systemic features, and the presence or absence of a family history are taken into account along with the presence of an *FBN1* gene mutation in the modified Ghent criteria. The diagnosis of Marfan syndrome remains a clinical one, but genetic testing of the *FBN1* gene can aid in the diagnosis when other criteria are not met, and over 600 mutations have been documented in the *FBN1* gene.

Syndromic FTAA

The phenotype of these syndromes is less distinctive compared to Marfan syndrome and therefore genetic testing has a more important role to play in diagnosis. Loeys-Dietz syndrome is caused by mutations in the TGF-β receptors 1 & 2, and is characterised by arterial tortuosity and aneurysm formation. There is a high risk of death before the age of 40 from thoracic or abdominal aortic dissection or intracranial haemorrhage and a high risk of adverse events in pregnancy, namely aortic dissection or

uterine rupture. Testing is therefore very important for this group of patients as there is a material impact on clinical management. Elective surgery is well tolerated in those patients who can be identified, with low mortality from aortic root replacement. Some patients may exhibit typical features including bifid uvula, wide-spaced eyes and feet deformities with arterial tortuosity, but in many patients a distinct phenotype is not evident.

Arterial tortuosity syndrome

This is a rare autosomal recessive condition. Mutations in the *SLC2A1O* gene result in this syndrome which is characterised by marked tortuosity in the branch vessels coming off the aorta, with stenosis and aneurysm formation, along with joint hypermobility, recessive jaw and skin elasticity.

Aneurysms-osteoarthritis syndrome

This is a rare autosomal dominant condition which accounts for approximately 2% of FTAA. Mutations in the *SMAD3* gene result in early onset osteoarthritis. Aggressive arterial disease is seen with bifid uvula, wide spaced eyes and hernia. Clinical management is similar to that for Loeys-Dietz syndrome.

Ehlers-Danlos Type IV syndrome

Rare mutations in the *COL3A1* gene are responsible for this syndrome with clinical features including spontaneous vascular and intestinal rupture. The condition is diagnosed with clinical and genetic features. Surgery is difficult and conservative therapy is recommended for all but life-threatening problems.

Genetic testing strategy*

In those with clear syndromic features, testing for phenotype-specific genes should be carried out: *FBN1* for Marfan syndrome, *TGFBR1 & 2* for Loeys-Dietz syndrome, *COL3A1* for Ehlers-Danlos Type IV syndrome, *SLC2A10* for arterial tortuosity and *SMAD3* for aneurysms-osteoarthritis syndrome. The strategy is more difficult in the case of non-syndromic cases. There may be some non-syndromic cases which have a mutation in TGF-β receptors 1 & 2, or other genes involved, such as the *ACTA2* gene. At present, testing does not usually extend beyond the *FBN1* gene and TGF-β receptor genes, and despite concerns around panel testing, it is useful in FTAA.

A genetic diagnosis allows a detailed management strategy for the proband, but also for potentially affected family members, with the benefit of clinical management at a lower disease threshold.

Panel testing

The current strategy of sequential single gene testing is time consuming and costly, relies upon phenotype/genotype correlation and is inconsistent amongst centres. Therefore panel testing represents an improved approach, which allows rapid results (within weeks), is useful for FTAA genotypes, and is particularly useful in non-syndromic cases of FTAA.

A genetic diagnosis allows a detailed management strategy for the proband, but also for potentially affected family members, with the benefit of clinical management at a lower disease threshold. In terms of limitations, the test may not pick up everything as it does not test non-coding regions, it cannot detect large insertions, and can be costly to set up.

Several panels exist for FTAA, including the 15 gene panel Harvard connective tissue disorders, which includes nine genes for aortic aneurysms with a 20% yield. The approved Manchester gene panel tests for nine genes, while the Newcastle commercial gene panel tests for 15 genes, with 99% coverage of the six most common genes plus an additional nine genes, with a 30% yield.

Genetic testing in this area may move from gene panels to whole exome and whole genome sequencing and the advantages and disadvantages of different approaches must be considered, including the coverage, complexity, the types of variants identified and the issue of VUS and IFs.

*see Appendix 2 for UKGTN testing criteria

100,000 Genomes Project

An aortic sub-domain of the cardiovascular Genomics England Clinical Interpretation Partnership (GeCIP) will focus on this clinical area and patients with the following conditions will be included:

- FTAA and dissection
- Thoracic aortopathy under 50 years old with no other risk factors
- Clinically diagnosed Marfan syndrome without *FBN1* mutation
- Loeys-Dietz syndrome and Loeys-Dietz syndrome like conditions
- Mutation negative congenital contractural arachnodactyly (Beals syndrome)

Patients must have been previously tested for genes specified within disease-relevant in silico panels along with standard local genetic testing and nationally commissioned testing for this phenotype. In addition, individual gene testing must have been conducted for variants with a diagnostic yield of over 10% for the phenotype.

A testing pathway was shown from De Backer *et al.*[8] which indicates the potential of NGS in finding a diagnosis in thoracic aortic (TAA) in a cohort of 264 patients, with mutations found in 34 patients. This relatively modest pickup rate included mutations found in patients who might not have been expected to be positive on the basis of their phenotypic features and had undergone previous testing. Therefore in some cases this approach was cost- and time efficient when compared to the prior testing which had been carried out.

Summary

Genetic testing allows for early genotyping for suspected hereditary aortopathy and informs the management strategy based on risk for patients and their families, as well as fulfilling the eligibility criteria for the 100,000 Genomes Project which provides the opportunity to find other disease pathways in FTAA syndromes.

[8] De Backer *et al.* Ann Cardiothoracic Surg 2013; 2(1): 73-82

4. Inherited cardiomyopathies 28 gene panel

Professor Hugh Watkins, University of Oxford

Introduction

Hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM) are mechanistically distinct conditions, although a number of genes are implicated in both conditions, namely the sarcomere genes. The mutations observed are mutually exclusive, with diametrically opposite biophysical properties, and ultimately clinical manifestations, observed for the two sets of mutations. Arrhythmogenic right ventricular cardiomyopathy (ARVC) does not share common causal genes with the other two conditions.

Over 1000 individual mutations are seen in HCM genes. The relative contribution of various genes was described, the most commonly implicated being *MYH7*, seen in 10-25% of cases, *MYBPC3* seen in 15-30% of cases, and *TNNT2* in 3-5% of cases. Panel testing is therefore useful as it includes genes which are less commonly but still definitively implicated in cardiomyopathies.

Cardiomyopathy panels

The Oxford cardiomyopathy panels include 16 genes for HCM. A subset of the genes on the panel can be guaranteed 100% coverage at a read depth of 30X, and will include infilling by Sanger if required. There are eight genes on the ARVC panel, and 28 genes on the DCM panel, with certain genes being more commonly implicated than others. The creation of panels is to some extent a dynamic process; as the aetiological evidence evolves there is pressure to remove some genes from the panel, and add in others.

Panel testing in HCM*

Advantages

Most variants implicated in HCM are seen in the three genes listed above, along with some rare variants in additional sarcomere genes. If panel tests include these additional sarcomere genes which are solidly implicated on the basis of family linkage data, then the yield of interpretable results is increased.

There are other disorders which are phenotypically slightly different, but it is useful to include variants associated with these related conditions. Therefore testing for variants in the *PRKAG2* gene, *GLA* gene (Fabry disease), *LAMP2* gene (Danon disease) and *FHL1* gene would be recommended; the diagnostic yield is low (around 1-3% each) but the clinical impact high (different natural history, inheritance patterns and treatment options).

The use of genetic testing is outlined in European Society of Cardiology (ESC) guidelines on the diagnosis and management of HCM[9]. The key benefits include the clinical efficacy of testing, the health economic advantages of discerning cases and unaffected individuals through familial screening, and the ability to examine a number of genes in conditions which exhibit phenocopies.

Panel testing has proved useful in identifying mutations in patients which would not have been suspected on the basis of their phenotype. In these cases a diagnosis is critical in informing clinical management, for example

[9] Elliot *et al.* Eur Heart J 2014; 35(39):2733-79

*see Appendix 3 for UKGTN testing criteria

enzyme replacement therapy or potential organ transplant, and to counsel the patient and wider family with knowledge of inheritance patterns.

Disadvantages

The use of wider gene panels leads to a deterioration in the signal to noise ratio in terms of causal versus non-causal variants. Evidence from a US group[10] shows the effects of widening the gene panel for DCM. When the gene panel is increased from five to 46 genes, the yield of class 4 and 5 variants increases (almost doubling), but the yield of class 3 variants increases to a much greater extent, with almost 60% of patients having a class 3 variant (variant of uncertain significance (VUS)). Such a high number of VUS presents significant problems for the clinician or genetic counsellor in dealing with the patient and their family.

Much of the work to identify causal variants in disease has been predicated on the assumption that a variant seen in a case is likely pathogenic if it is absent in 200 unaffected controls, and so is not a 'polymorphism'. However, it is now known that the occurrence of rare and extremely rare variants in unaffected individuals is 10-100 times greater than previously thought. Therefore rarity as a criteria is not sufficient to suggest pathogenicity. Absence of a variant in normal controls, together with relatively weak measures of *in silico* prediction, has been considered sufficient to support the case for pathogenicity. However, in the absence of familial linkage data, these are now known not to be reliable indicators of pathogenicity.

Therefore, many variants are over-called in the literature, particularly the research literature, with around 10-20% of variants over-called for HCM and more for other disorders. It is also clear that some genes create more 'noise' than others in terms of VUS. The result is that some genes included in diagnostic panels may have no causal link with the disease.

Implications of wider testing

The signal to noise ratio deteriorates with lower prior probability, which can result from widened testing and testing individuals with indistinct phenotypes. Therefore, increasing the number of genes on the panel increases the amount of noise and is even more apparent with whole exome and whole genome approaches to testing. Testing patients without a clear phenotype also reduces the chance that rare variants observed are pathogenic.

There is a temptation to look at a wider number of genes simply because they are available on a panel. This is not to be recommended in a diagnostic setting for the reasons outlined above. However, in a research setting it may be useful to evaluate additional genes in this respect. There are concerns that clinicians and laboratories could be tempted to test just because it is possible, without any diagnostic advantage, and with the downside of increased VUS. Therefore it is important not to test the wrong gene for the wrong condition.

The importance of annotation and collation of co-segregation data

Annotation of domains and classes of variants is critically important to inform the likely pathogenicity of variants found, and in helping to identify the type of variant and domain to look for. For example, analysis of DCM and HCM variants shows an enrichment around the globular motor head of the myosin heavy chain protein and much less around the rod domain of the protein. Similarly, where a laboratory has evidence of co-segregation, or lack of, regarding a specific variant, this information is of key importance and mechanisms for sharing of curated data are needed.

Summary

The search for an underlying genetic cause of disease is probabilistic, and therefore relies heavily on the prior likelihood of the condition. Panels must include curated genes that have been shown to be pathogenic based on linkage studies or with robust burden tests. The process can be improved by increased knowledge around the domains and classes of variants, which requires experts dedicated to intensive study of a small number of genes. Global data-sharing is also key to uncovering the significance of novel variants. Above all, it is critically important to test the right genes for the right condition.

[10] Pugh *et al*. Genet Med 2014; 16(8): 601-8

5. Familial hypercholesterolaemia gene panel testing: closing the gap in ascertainment

Dr Maggie Williams, Bristol Genetics Laboratory

Introduction

Familial hypercholesterolaemia (FH) is a dominant disease which is estimated to affect around 1 in 500 people in the UK, although the figure may be nearer to 1 in 200, with around 1 in 1,000,000 people having severe homozygous FH. Currently, around 15% of affected individuals are diagnosed, with the hope that high throughput testing in the form of NGS technologies will radically improve case ascertainment in a cost-effective way. The disease is characterised by premature atherosclerosis, increased risk of coronary heart disease, angina, heart attack, stroke, pain on walking and other phenotypic features such as tendon xanthoma, xanthelasma and cornea arcus.

Benefits of testing for FH

The risk of clinical sequelae are considerably reduced with statin therapy or LDL-C apheresis for homozygous FH cases, and several new therapies are in development. The main benefit of a genetic diagnosis is that cascade testing can be offered to first, second and third degree relatives to identify affected individuals and begin appropriate clinical management early.

Diagnosis

A lipid screen measuring total and LDL cholesterol can be used to identify possible cases of FH. Simon Broom diagnostic criteria or modified Dutch criteria are used to identify definite and possible FH cases based on biochemistry, physical signs, family history and DNA evidence. Recently published evidence from a centre in Wales described the experience of using modified Dutch criteria to score patients and allows for weighting of early-onset disease and additional relatives[11].

Although the lipid screen is a relatively easy and inexpensive test, it is not a sufficient standalone diagnostic tool because of the overlap in cholesterol levels in the affected and unaffected populations, which increases with age. Therefore testing in this way does not provide the unambiguous result which is required for a cascade programme.

Definite FH is defined by specific biochemical features, tendon xanthomas, evidence in first and second degree relatives and DNA evidence. Possible FH is considered on the basis of biochemical features along with a family history of myocardial infarction, or raised total cholesterol in a relative.

The Dutch and modified Dutch scoring criteria state that:

"Diagnosing patients on the basis of cholesterol alone is problematic due to the overlap in total cholesterol levels between affected and non-affected individuals, and makes genetic testing valuable for giving an unambiguous result."

The main benefit of a genetic diagnosis is that cascade testing can be offered to first, second and third degree relatives to identify affected individuals and begin appropriate clinical management early.

[11] Haralambos et al. Atherosclerosis 2015; 240(1): 190-6

Genetics of FH

Mutations in four key genes associated with cholesterol metabolism are responsible for FH. These include the LDL receptor gene, the *APOB* gene encoding a cofactor ligand which helps to bind LDL to the receptor, the *PKSC9* gene which is involved in LDL receptor recycling, and a mutation in the *LDLRAP1* gene, seen in a recessive form of FH, which is involved in receptor/ligand internalisation. In 10-15% cases the genetic cause is unknown.

In a cohort of over 900 patients referred to the Bristol genetic testing service, a wide spectrum of variants was seen, with 61% of variants seen only once, therefore necessitating comprehensive screening of the key genes. Copy number variants are also implicated in FH and around 5-8% cases are caused by large deletions or duplications, with the former being more common. MLPA testing has traditionally been used to pick up these classes of mutation.

In the past few years, exome sequencing has been employed to determine novel genetic causes of FH and this has detected novel variants in the *APOB* gene. A research team led by Steve Humphries has also identified variants in the promoter region of the *LDLR* gene which contribute to FH[12]. Novel genes are also being identified which may have an association with raised cholesterol levels *e.g. STAP1* gene.

FH is included in the list of secondary findings which may be fed back to patients as part of the 100,000 Genomes Project. A search for new FH-causing genes will also form part of the cardiac Genomics England Clinical Interpretation Partnership (GeCIP) proposal.

Recommendations for high throughput testing

As well as finding a wide range of variants, NGS can offer the high throughput testing required to deliver an effective cascade testing programme. NICE has issued guidance on testing for FH, along with the Department of Health's 2013 Cardiovascular disease outcomes strategy which states that the current diagnosis rate should be improved from 15 to 50% of the estimated FH cases in the UK. The BHF has invested in excess of £1.5 million for a cascade testing programme for England, with FH nurses in post and an initial aim of providing 50% of England with access to the new testing regime.

FH genetic testing

Previously FH testing involved targeted mutation testing, for around 20 mutations using amplification refractory mutation system analysis (ARMS) or chips such as LipoChip, or Iplex testing covering 56 mutations. Testing has then evolved through automated sequencing with MLPA and ultimately NGS, with a progressive reduction in costs and increase in throughput. NGS has the advantage of being a cost-effective, high throughput method which can lead to faster diagnosis.

FH assay

The NGS haploplex assay uses Illumina sequencing and Agilent chemistry, and took around two years to develop, with extensive validation. Bioinformatic analysis is carried out using a variety of validated bespoke and online tools.

Currently 16 samples can be analysed simultaneously with this set to increase to 48 and 96 samples. At £250 the test represents a substantial reduction in costs, all

[12] Khamis *et al*. Eur J Hum Genet 2015; 23(6):790-5

genes can be included and CNVs are picked up so no secondary test is required. Polymorphisms associated with statin myopathy have also been included, which can be useful for FH patients who have adverse reactions to statin therapy. The ability to detect SNPs associated with polygenic FH and variants in the *STAP1* gene are the latest additions to the panel.

Results from diagnostic referrals

Diagnostic referrals are increasing, and data from a cohort of 1010 patients referred for testing shows a higher detection rate of class 4 and class 5 variants seen with NGS, with 320 positive patients (32%) in comparison to 30.5% of patients prior to NGS. A wider array of pathogenic variants are seen, with 181 different pathogenic mutations identified so far, mostly point mutations, with some small indels and 20 patients with deletions and two with duplications. A larger number of homozygous cases have been identified and some compound heterozygotes (*LDLR/APOB*) with milder phenotypes. Ten homozygous FH cases were identified, mostly involving the *APOB* gene, including one case from a consanguineous family who was homozygous for three *LDLR* mutations.

The greater sensitivity of NGS has impacted on the pickup rate, detecting a proportion of cases which had been missed by previous methods, particularly rare variants in the *APOB* gene. However the increased sensitivity has also led to a number of VUS being identified.

Case studies: patients identified through NGS assay

A case study was described involving a 58 year old woman who was referred with raised cholesterol and a suggestive family history. Genetic testing revealed a mutation in *PCSK9*, which has also been described in a small Italian study. Cascade testing identified some other family members who were positive for this mutation, who would have been missed prior to the application of NGS.

A further case study involved a 59 year old man with raised cholesterol and extensive family history of CVD, in whom a mutation in the *APOB* gene was found, having previously been reported in the literature[13]. Cascade testing has been offered to the family.

Variants of unknown significance (VUS)

Data from referrals show that 6% of patients were found to have VUS with 27 found in the *APOB* gene, 10 in the *PCSK9* gene and 15 in the *LDLR* gene. The proportion of variants which are regarded as VUS is diminishing as knowledge increases. Close collaboration with research groups (UCL and University of Wales, Cardiff) is critically important to this process, with functional analysis and segregation studies key, along with active data sharing by testing laboratories.

Data showing the detection rate for different referral types from different UK centres was shown and reflects to some extent the selection criteria employed before referral, with an overall positive detection rate of 32%.

Testing criteria* and case ascertainment

The UKGTN testing criteria and genetic test application describes the recommended approach to testing for FH, and various initiatives are looking at improving case ascertainment including referral protocols in lipid clinics. NHS England are reviewing the care pathway, the timing of tests and criteria for referral. In addition, NICE has carried out a review of guidance in this area. Engaging with GPs and other cohorts is also important to drive forward better referral and diagnosis rates.

Cascade testing

BHF funding is supporting the PASS clinical system based on Welsh and Dutch FH cascade testing programmes, and the aim is that PASS will be implemented in all lipid centres in England. The system collates clinical, biochemical, pedigree, treatment and genetic information and manages clinical appointments and follow up letters. There is also an active electronic workflow between clinics and laboratories.

[13] Motazacker *et al.* Eur Heart J 2012; 33(11): 1360-6

*see Appendix 4 for UKGTN testing criteria

6. The role of whole genome sequencing in cardiovascular disorders

Professor Bernard Keavney, University of Manchester

Introduction

The unmet diagnostic need in cardiovascular disease is reflected in the inclusion of a cardiovascular domain within the 100,000 Genomes Project. This project is focusing on rare diseases for which there is likely to be a single gene cause. There are many of these in cardiovascular medicine; the list of conditions which will be included in the project includes cardiomyopathies, hyperlipidaemias, aortopathy and aortic dissection amongst others.

Panel tests do not provide a diagnosis for all families, and it is therefore useful to consider the diseases for which whole exome or whole genome sequencing might be useful diagnostic tools. In general these will be conditions with clear Mendelian inheritance, one such diagnostic group may be congenital heart disease, which has been included in the 100,000 Genomes Project. Although CHD patients are a highly heterogeneous group, with evidence of polygenic inheritance in most cases, a subgroup of patients may have disease caused by a single mutation, or a highly penetrant CNV. Studies so far have shown that the burden of disease attributable to *de novo* copy number variants may be 5-10% in apparently sporadic cases, with a further 5-10% due to *de novo* single nucleotide variants.

Panel tests and whole exome/whole genome sequencing

The trajectory from single gene testing to panel testing and whole exome and whole genome sequencing was described. Various gene panels such as the Oxford 28 gene cardiomyopathy panel, Manchester 62 gene sudden cardiac death panel, and Bristol 73 gene paediatric cardiomyopathy panels were referenced, along with the Illumina Trusight cardiopanel for research use, which can examine 174 genes at a cost of around $1 per gene. The approach taken will depend on the detailed patient phenotype, family history and the likely nature of the disease causing variants, as well as consideration of variants of uncertain significance (VUS) and incidental findings (IFs). An important danger is over interpretation of VUS, as the genomic 'search space' increases in size. Cost is becoming proportionally less critical as the costs of reagents and equipment begin to converge across panel testing, exomes and whole genomes. However there will remain important differences between the approaches. Volumes of data that are generated and the associated management issues (particularly for whole genomes) are very different. Interpretation of VUS is an increasing challenge as larger segments of the genome are sequenced; it is important to recognise that there are differences in clinical science expertise between laboratories for particular genes in which some laboratories have many years of experience in interpretation. A larger scale experiment will reveal more incidental findings, and there remains robust debate internationally regarding which of these to feedback and to whom.

Although CHD patients are a highly heterogeneous group, with evidence of polygenic inheritance in most cases, a subgroup of patients may have disease caused by a single mutation, or a highly penetrant CNV.

Indications for different testing strategies were described. Increasing levels of genetic heterogeneity coupled with indistinct phenotypic features and an important role for *de novo* variation would point towards exome and genome sequencing, with gene panels representing an intermediate option. Studies so far have shown the value of whole exome sequencing (WES) or whole genome sequencing (WGS) in patients with difficult-to-resolve phenotypes who turn out to carry pathological variants for two different diseases- practically all clinical WES/WGS studies have found such patients. Also, WES/WGS approaches have discovered mutations in known disease genes in patients with phenotypes that are atypical for the condition (*e.g.* Noonan's syndrome). WES/WGS may be particularly useful in these situations. Examples of disorders in which a single gene testing approach is optimal would include *CFTR* testing for cystic fibrosis, trinucleotide repeat disorders, and diseases such as Prader-Willi and Angelman syndromes; gene panels would be anticipated to continue to be useful in testing for muscular dystrophies, RASopathies and cardiomyopathy; whilst WES and WGS might be applied for conditions such as intellectual disability.

Differences between exome and genome sequencing

Exome sequencing provides higher coverage at lower cost, and is more widely available, with less challenging data management. Eighty percent of variants causing Mendelian conditions are believed to be exonic. Genome sequencing is better for structural variants, has more uniform coverage and includes regulatory regions of the genome. It also includes testing of common variants which may be useful in the context of complex disease and pharmacogenetics.

Studies on whole exome sequencing and whole genome sequencing

Although WES and WGS are most typically employed in diseases with clear Mendelian inheritance, other conditions also show promise, for example congenital heart disease. Details of a study were presented in which WES was carried out on samples from 364 severe congenital heart disease trios and 264 control trios, and an odds ratio of 7.5 was found for *de novo* mutations in genes strongly expressed in the mouse developing heart[14]. In particular, genes involved in *H3K4* and *H3K27* methylation were over-represented. This study suggested that hundreds of genes are involved in the aetiology of congenital heart disease and around 10% of cases may arise from *de novo* single nucleotide variants. If confirmed by future studies, this would lead to a genetic diagnosis being possible in a substantial fraction of CHD patients.

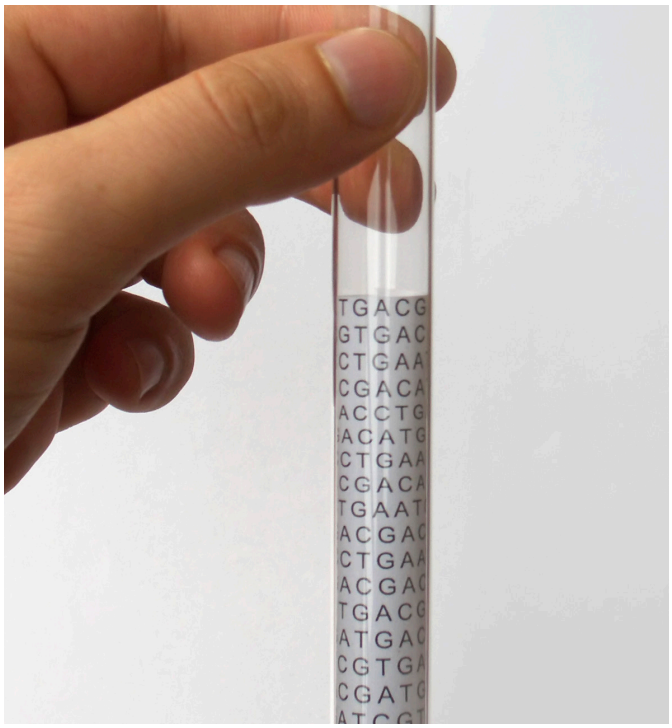
A further study by Gilissen *et al.*[15] was described in which genome sequencing was used to identify the cause of severe intellectual disability. WGS identified a causal mutation in a substantial proportion (42%) of 50 patients when both array-CGH and WES had not resulted in a diagnosis, and these were *de novo* mutations. Therefore, if there is a suspicion that *de novo* mutations are involved in the condition, then WGS may have a valuable role to play.

WES and WGS limitations

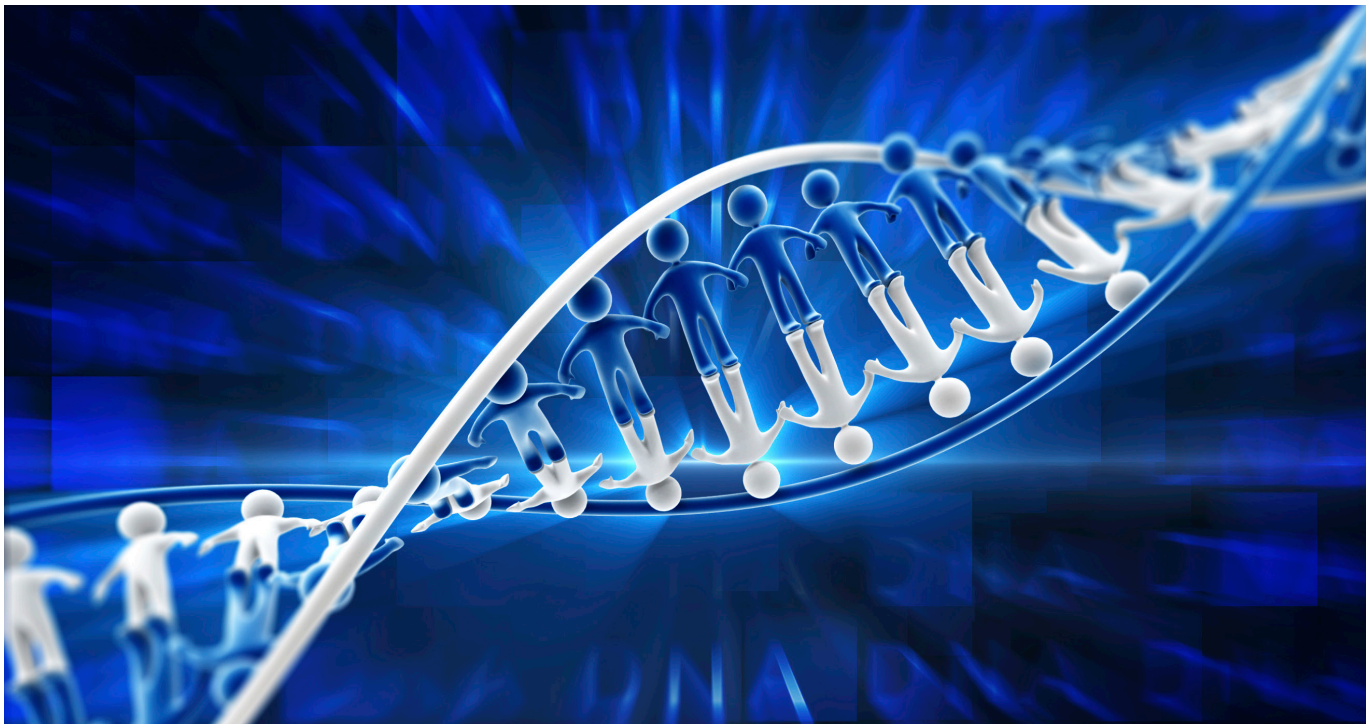
WES and WGS using short read platforms do not detect all DNA variant types well. Trinucelotide repeats are not well detected, along with copy number variants as are seen in some deletions associated with cardiac disease (*e.g.* 22q11.2 deletion) and duplications (*e.g.* 1q21.1 duplication). Larger indels (between 10 and 1000bp) are not always accurately detected with WES and WGS, along with structural variants such as chromosomal translocations. WES and WGS may not be the most suitable method for aneuploidy detection and will not provide information on epigenetic alterations.

Variants of uncertain significance (VUS) and incidental findings (IFs)

Between 100 and 500 private protein-altering variants will typically be identified in an individual with WES based testing, of which around 40-100 will be human gene mutation database (HGMD) disease-causing variants, with 100 heterozygous loss of function (LOF) variants and 20 homozygous LOF variants. Therefore the interpretive complexity is substantially increased by doing WES or WGS based testing.



[14] Zaidi *et al.* Nature 2013; 498(7453): 220-3
[15] Gilissen *et al.* Nature 2014; 511(7509): 344-7



Approaches to feeding back incidental findings vary. The American College of Medical Genetics (ACMG) produced a list of 56 medically actionable genes and the complexity of this area is reflected in the revisions to this list and ongoing debate in this area. Data from NHLBI exome sequencing project (ESP) in 2015 considered the distribution of variants in 112 medically actionable genes (which included the ACMG 56 genes) and showed potentially reportable variants were present in 2% of people with European ancestry and 1.1% of people with African ancestry (the proportion was lower when considering only the ACMG 56 gene list: 1.6% and 1.0% respectively).

There remains a substantial amount of heterogeneity in variant classification between laboratories and the process will be heavily dependent on specialist expertise with particular genes and more extensive databases of variants with better curation.

Preparing to order an exome and genome in the clinical context (guidance from Biesecker and Green 2014[16])

Appropriate candidate gene tests should be carried out initially, and thorough information must be gathered on family history with a systematic approach to phenotyping. Literature and database searches should be conducted to see if this would inform the approach to testing and informed consent is vitally important.

Examples of clinical studies using WES or WGS

1. A study by the Medical College of Wisconsin[17] was described involving a clinical WGS testing programme in which 23 paediatric and two adult patients were tested, resulting in seven diagnoses and seven possible diagnoses. The complexities centred around incorporating WGS testing into clinical practice, in terms of the logistics, the interpretation by clinicians and the use of incidental findings by patients and their families.

2. A WES study was conducted at Baylor College of Medicine by Yang *et al.*[18] in 250 probands, 80% of whom were children with neurological phenotypes. All had had prior array-CGH, metabolic screening and single gene sequencing or a combination of these tests. Mutations were found in 25% of patients, half being autosomal dominant mutations and around 83% being *de novo* mutations. In 30 of the 250 patients the findings, involving 16 genes, medically actionable incidental findings were discovered. Four patients were diagnosed with Noonan spectrum disorders: one had recognised clinical features but the mutation was in a previously unreported gene. Three of the patients had atypical clinical phenotypes but mutations in known genes which had not been tested, as the phenotype was atypical, and this is an important area in which NGS can contribute.

[16]Biesecker and Green N Engl J Med 2014; 370(25): 2418-25
[17]Jacob *et al.* Sci Transl Med 2013; 5(194): pp194cm5
[18]Yang *et al.* N Engl J Med 2013; 369(16): 1502-11

3. FORGE Canada consortium[19]: WES was carried out in 264 patients with congenital, paediatric, likely monogenic disorders. The diagnostic likelihood varied according to the inherent properties of the case. The results stratified in this way show that for multiple unrelated individuals or multiple families with highly recognised disorders, mutations were found in novel genes in around 50% of such patients, with most of the remaining patients being diagnosed with mutations in known genes.

   In consanguineous families a slightly lower pickup rate in novel genes was observed. In autosomal dominant families (even those with four or five informative meioses) very few mutations in novel genes were observed, with a less than 40% pickup rate in known genes.

   In non-consanguineous families with two or more affected siblings the pickup rate was slightly higher, whilst testing of single affected individuals with no family history resulted in a very low pickup rate. In this study, 67 novel genes were identified (41 genes validated and 26 strong candidate genes). Mutations in 95 known genes were found and these mutations often broadened the disease phenotype. Some patients were found to have more than one rare disease, and 118 of all the cases remained undiagnosed.

4. Oxford WGS 500 study[20]: WGS was used in testing of 156 cases with Mendelian and immunological disorders where previous genetic screening was negative. Some important technical factors were identified in the data analysis. Joint calling in family members eliminated 90% of putative *de novo* mutations, reducing this from 32.1 to 2.6 per trio. Variants were also filtered according to whether they were present in other probands in the study with unrelated phenotypes, which reduced the number of homozygous variants with a frequency of less than 0.5% from 80.8 to 1.5 per family. In addition, multiple annotation approaches were taken.

The diagnosis rate varies across different phenotypes. Mutations in known genes were found for all patients with LQT syndrome and no novel genes identified. In contrast, for adult onset dominant cardiovascular diseases, for example, familial dilated cardiomyopathy, familial cardiomyopathy with repolarisation abnormalities and familial cardiomyopathy with mixed features, no causal genes were identified- indeed, multiple good candidates emerged but a very large amount of functional work including the generation of animal models for each of the genes would be required to make progress. Clearly in the clinical scenario this is not feasible

Overall there was a 21% yield in the study which was highest for recessive and *de novo* mutations (57% of trios). The lowest diagnostic rates were seen for adult onset dominant conditions. No diagnostic success was seen at the extremes of phenotype, for example young onset cases of polygenic conditions. Four variants were found in 156 families which were reportable under the ACMG's approach to IFs.

In terms of confirming pathogenicity, a combination of candidacy, predicted function, frequency and conservation was not always sufficient. Details of familial transmission were also needed along with functional data, *de novo* status and/or additional patients. Therefore it seems that high throughput functional investigation platforms will be critical to the success of clinical WES and WGS based testing, for example CRISPR animal models and human embryonic stem cells (hESC)/induced pluripotent (iPSC) based models.

100,000 Genomes Project

It was stated for information that a cardiovascular domain of the Genomics England Clinical Interpretation Partnership (GeCIP) has been designated, and an estimated 1000 trios will be available for cardiovascular phenotypes. Colleagues interested to participate in the GeCIP domain's activities are invited to email Bernard Keavney (bernard.keavney@manchester.ac.uk).

Summary

Single gene testing, panel testing and WES/WGS based testing all have a role to play in diagnosing cardiovascular disease. The impact of lower costs may result in a move towards a single streamlined WGS workflow but this appears some time off yet. A shift in emphasis is likely to see a move away from differential diagnosis pre-WGS to post-WGS diagnostic assessment, with a periodic re-examination of a patient's genome throughout their lifetime.

Next generation phenotyping is critical to the success of next generation sequencing, along with access to appropriate genomics training for cardiologists. Close cooperation between clinical genetics and diagnostic laboratory colleagues is fundamentally important, and careful attention must be paid to ethical, legal and social issues to ensure that clinicians are acting as advocates for their patients' needs.

[19] Beaulieu *et al.* Am J Hum Genet 2014; 94(6): 809-17
[20] Taylor *et al.* Nat Genet 2015; 47(7): 717-26

Discussion and conclusion

Discussion

Whilst information from wider testing may be useful in a research context, expert interpretation is critical and the potential to cause harm to patients in the form of VUS and IFs means that a distinction must be made between clinical and research approaches to testing. This was echoed in the example of LQT syndrome testing where the inclusion of more genes on the panel may have led to the belief that diagnostic rates have improved, when in fact some diagnoses were misappropriated to 'noisy' genes with many variants which are not pathogenic. Looking forward, the success of these new technologies in a clinical context will be critically dependent on a high throughput functional pipeline incorporating NGS technologies and high quality modelling of organ pathology.

Conclusion

The two sessions on genetic testing in cardiovascular conditions were well attended, reflecting the growing interest amongst cardiology professionals in these diagnostic technologies, and the increased access to genetic testing in areas of mainstream medicine. The greater use of such testing has resulted in significantly improved diagnostic rates, as highlighted in presentations on the use of gene panels in the investigation of sudden cardiac death, and through the use of NGS technologies in FH testing. However, some concerns remain regarding the equity of access to such testing nationwide, with some divergence in panel design amongst different laboratories. Therefore further work is needed to establish the framework to assess and inform the optimal composition of panel tests. This is crucial to providing equitable and safe testing for patients and ensuring maximum clinical utility.

Appendix 1

UKGTN testing criteria for **sudden cardiac death syndromes**

1. Arrhythmia/cardiac arrest (BCL) 21 gene panel
2. Arrhythmogenic right ventricular cardiomyopathy (ARVC) 6 gene panel
3. Brugada syndrome 6 gene panel
4. Long QT syndrome 12 gene panel
5. Catecholaminergic polymorphic ventricular tachycardia (CPVT) RYR mutation analysis
6. Molecular autopsy 57 gene panel
7. Hypertrophic cardiomyopathy 22 gene panel

UKGTN Testing Criteria

Test name: **Arrhythmia/Cardiac Arrest (BCL) 21 Gene Panel**	
Approved name and symbol of disorder/condition(s): See Appendix 1	**OMIM number(s):**
Approved name and symbol of gene(s): See Appendix 1	**OMIM number(s):**

Patient name:	**Date of birth:**
Patient postcode:	**NHS number:**
Name of referrer:	
Title/Position:	**Lab ID:**

Referrals will only be accepted from one of the following:	
Referrer	**Tick if this refers to you.**
Consultant Clinical Geneticist	
Consultant Cardiologist	

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:	
Criteria	**Tick if this patient meets criteria**
In individuals with idiopathic ventricular tachycardia (VT) or resuscitated (VF) cardiac arrest without known cause	
In individuals with idiopathic ventricular tachycardia (VT) or resuscitated (VF) cardiac arrest without known cause and family history of sudden cardiac death	

Additional Information:

For panel tests:
At risk family members where familial mutation is known do not require a full panel test but should be offered analysis of the known mutation

If the sample does not fulfil the clinical criteria or you are not one of the specified types of referrer and you still feel that testing should be performed please contact the laboratory to discuss testing of the sample.

Arrhythmia/cardiac arrest (BCL) 21 gene panel

Genes in panel test and associated conditions
Rows that are highlighted in yellow show where the gene is currently being fully analysed in the context of a single separate UKGTN test

HGNC standard name and symbol of the gene	HGNC number	OMIM number	OMIM standard name of condition and symbol	Mode of inheritance	OMIM number	Evidence of association between gene(s) and condition	% of horizontal coverage of gene	MLPA	Comments
AKAP9	HGNC:379	*604001	Long QT syndrome-11	AD	611820	PubMed: 18093912	100%	No	Not available in UKGTN
ANK2	HGNC:493	*106410	Cardiac arrhythmia, ankyrin-B-related Long QT syndrome-4	AD AD	600919 600919	PubMed: Various PubMed: Various	100%	No	Fully analysed as a single separate UKGTN test
CACNA1C	HGNC:1390	*114205	Brugada syndrome 3	AD	611875	PubMed: Various	100%	No	Not available in UKGTN
CALM1	HGNC:1442	*114180	Ventricular tachycardia, catecholaminergic polymorphic, 4	AD	614916	Pubmed Various	100%	No	Not available in UKGTN
CASQ2	HGNC:1513	*114251	Ventricular tachycardia, catecholaminergic polymorphic, 2	AR	611938	PubMed: Various	100%	No	Not available in UKGTN
GPD1L	HGNC:28956	*611778	Brugada syndrome 2	AD	611777	PubMed: Various	100%	No	Not available in UKGTN
HCN4	HGNC:16882	*605206	Brugada syndrome 8	AD	613123	PubMed: 19165230	100%	No	Not available in UKGTN
KCNE1	HGNC:6240	*176261	Long QT syndrome-5	AD	613695	PubMed: Various	100%	No	Available in UKGTN panel test
KCNE2	HGNC:6242	*603796	Atrial fibrillation, familial, 4 Long QT syndrome-6	AD AD	611493 613693	PubMed: Various PubMed: 10219239	100%	No	Available in UKGTN panel test
KCNE3	HGNC:6243	*604433	Brugada syndrome 6	AD	613119	PubMed: Various	100%	No	Not available in UKGTN
KCNH2	HGNC:6251	*152427	Long QT syndrome-2 Short QT syndrome-1	AD AD	613688 609620	PubMed: Various PubMed: Various	100%	No	Available in UKGTN panel test
KCNJ2	HGNC:6263	*600681	Atrial fibrillation, familial, 9 Short QT syndrome-3	AD AD	613980 609622	PubMed:15922306 PubMed:15761194	100%	No	Available in UKGTN panel test
KCNJ5	HGNC:6266	*600734	Long QT syndrome 13	AD	613485	PubMed: 20560207	100%	No	Not available in UKGTN

KCNQ1	HGNC:6294	*607542	Atrial fibrillation, familial, 3 Long QT syndrome-1 Short QT syndrome-2	AD AD/AR AD	607554 192500 609621	PubMed: 12522251 PubMed: Various PubMed: 15159330	100%	No	Available in UKGTN panel test
RYR2	HGNC:10484	*180902	Arrhythmogenic right ventricular dysplasia 2 Ventricular tachycardia, catecholaminergic polymorphic, 1	AD AD	600996 604772	PubMed: Various PubMed: 11159936	100%	Yes – selected exons	Fully analysed in the context of a single separate UKGTN test
SCN1B	HGNC:10586	*600235	Atrial fibrillation, familial, 13 Brugada syndrome 5	AD AD	615377 612838	PubMed: Various PubMed: 18464934	98%	No	Not available in UKGTN
SCN3B	HGNC:20665	*608214	Brugada syndrome 7	AD	613120	PubMed: 20031595	100%	No	Not available in UKGTN
SCN4B	HGNC:10592	*608256	Long QT syndrome-10	AD	611819	PubMed: 17592081	100%	No	Not available in UKGTN
SCN5A	HGNC:10593	*600163	Atrial fibrillation, familial, 10 Brugada syndrome 1 Cardiomyopathy, dilated, 1E Long QT syndrome-3 Ventricular fibrillation, familial, 1	AD AD AD AD AD	614022 601144 601154 603830 603829	PubMed: Various PubMed: Various PubMed: Various PubMed: Various PubMed: 10940383	100%	No	Available in UKGTN panel test
SNTA1	HGNC:11167	*601017	Long QT syndrome 12	AD	612955	PubMed: 18591664	99%	No	Not available in UKGTN
TRDN	HGNC:12261	*603283	Ventricular tachycardia, catecholaminergic polymorphic, 5, with or without muscle weakness	AR	615441	Pubmed Various	100%	No	Not available in UKGTN

UKGTN Testing Criteria

Test name: **Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC) 6 Gene Panel**	
Approved name and symbol of disorder/condition(s): See Appendix 1	**OMIM number(s):**
Approved name and symbol of gene(s): See Appendix 1	**OMIM number(s):**

Patient name:	**Date of birth:**
Patient postcode:	**NHS number:**
Name of referrer:	
Title/Position:	**Lab ID:**

Referrals will only be accepted from one of the following:	
Referrer	**Tick if this refers to you.**
Consultant Clinical Geneticist	
Consultant Cardiologist	

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:	
Criteria	**Tick if this patient meets criteria**
TWO of : **1.** RV dilatation, functional impairment, or localised RV aneurysm, in the absence of similar LV dysfunction. **2.** Fibrofatty replacement of myocardium seen on biopsy **3.** ECG shows prolongation of QRS focally in leads V1-V3 **4.** Family history of definite ARVC detected at autopsy/surgery	
OR ONE of above, AND ONE OR MORE OF : **4.** Mild RV dilatation, impairment, or focal RV hypokinesis in presence of normal LV. **5.** ECG shows inverted T waves in V2, V3, in absence of RBBB, OR shows signal-averaged late potential. **6.** LBBB-type VT, OR frequent Vent.ectopics (>1000/24hrs) **7.** Close F.Hist. of sudden cardiac death <35yrs, suspected as ARVD	
OR NONE OF 1-3, but ALL of 4-7.	

Additional Information:

For panel tests:

At risk family members where familial mutation is known do not require a full panel test but should be offered analysis of the known mutation

If the sample does not fulfil the clinical criteria or you are not one of the specified types of referrer and you still feel that testing should be performed please contact the laboratory to discuss testing of the sample.

Approval Date: Sept 2014

Arrhythmogenic right ventricular cardiomyopathy (ARVC) 6 gene panel

Genes in panel test and associated conditions
Rows that are highlighted in yellow show where the gene is currently being fully analysed in the context of a single separate UKGTN test

HGNC standard name and symbol of the gene	HGNC number	OMIM number	OMIM standard name of condition and symbol	Mode of inheritance	OMIM number	Evidence of association between gene(s) and condition	% of horizontal coverage of gene	MLPA	Comments
DSC2	HGNC:3036	*125645	Arrhythmogenic right ventricular dysplasia 11	AD	610476	PubMed: Various	100%	Yes – selected exons	Fully analysed in a UKGTN panel test
DSG2	HGNC:3049	*125671	Arrhythmogenic right ventricular dysplasia 10 Cardiomyopathy, dilated, 1BB	AD AD	610193 610193	PubMed: Various PubMed: Various	100%	Yes – selected exons	Fully analysed in a UKGTN panel test
DSP	HGNC:3052	*125647	Arrhythmogenic right ventricular dysplasia 8	AD	607450	PubMed: Various	100%	Yes – selected exons	Fully analysed in a UKGTN panel test
JUP	HGNC:6207	*173325	Arrhythmogenic right ventricular dysplasia 12	AD	611528	PubMed: 17924338	100%	Yes – selected exons	Not available in UKGTN
PKP2	HGNC:9024	*602861	Arrhythmogenic right ventricular dysplasia 9	AD	609040	[PubMed: 15489853	100%	Yes – selected exons	Fully analysed in a UKGTN panel test
TMEM43	HGNC:28472	*612048	Arrhythmogenic right ventricular dysplasia 5	AD	604400	PubMed: Various	100%	No	Not available in UKGTN

Approval Date: Sept 2014

UKGTN Testing Criteria

Test name: **Brugada Syndrome 6 Gene Panel**	
Approved name and symbol of disorder/condition(s): See Appendix 1	**OMIM number(s):**
Approved name and symbol of gene(s): See Appendix 1	**OMIM number(s):**

Patient name:	**Date of birth:**
Patient postcode:	**NHS number:**
Name of referrer:	
Title/Position:	**Lab ID:**

Referrals will only be accepted from one of the following:	
Referrer	**Tick if this refers to you.**
Consultant Clinical Geneticist	
Consultant Cardiologist	

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:	
Criteria	**Tick if this patient meets criteria**
Index case with ECG consistent with Brugada syndrome **AND**	
A family history consistent with autosomal dominant inheritance **OR**	
A personal history of syncope without warning and/or aborted cardiac arrest.	

Additional Information:

For panel tests:
At risk family members where familial mutation is known do not require a full panel test but should be offered analysis of the known mutation

If the sample does not fulfil the clinical criteria or you are not one of the specified types of referrer and you still feel that testing should be performed please contact the laboratory to discuss testing of the sample.

Approval Date: Sept 2014

Brugada syndrome 6 gene panel

UK Genetic Testing Network

Genes in panel test and associated conditions
Rows that are highlighted in yellow show where the gene is currently being fully analysed in the context of a single separate UKGTN test

HGNC standard name and symbol of the gene	HGNC number	OMIM number	OMIM standard name of condition and symbol	Mode of inheritance	OMIM number	Evidence of association between gene(s) and condition	% of horizontal coverage of gene	MLPA	Comments
GPD1L	HGNC:28956	*611778	Brugada syndrome 2	AD	611777	PubMed: Various	100%	No	Not available in UKGTN
HCN4	HGNC:16882	*605206	Brugada syndrome 8	AD	613123	PubMed: 19165230	100%	No	Not available in UKGTN
KCNE3	HGNC:6243	*604433	Brugada syndrome 6	AD	613119	PubMed: Various	100%	No	Not available in UKGTN
SCN1B	HGNC:10586	*600235	Atrial fibrillation, familial, 13 Brugada syndrome 5	AD AD	615377 612838	PubMed: Various PubMed: 18464934	98%	No	Not available in UKGTN
SCN3B	HGNC:20665	*608214	Brugada syndrome 7	AD	613120	PubMed: 20031595	100%	No	Not available in UKGTN
SCN5A	HGNC:10593	*600163	Atrial fibrillation, familial, 10 Brugada syndrome 1 Cardiomyopathy, dilated, 1E Long QT syndrome-3 Ventricular fibrillation, familial, 1	AD AD AD AD AD	614022 601144 601154 603830 603829	PubMed: Various PubMed: Various PubMed: Various PubMed: Various PubMed: 10940383	100%	No	Available in UKGTN panel test

Approval Date: Sept 2014

UKGTN Testing Criteria

Test name: **Long QT Syndrome (LQT) 12 Gene Panel**	
Approved name and symbol of disorder/condition(s): See Appendix 1	**OMIM number(s):**
Approved name and symbol of gene(s): See Appendix 1	**OMIM number(s):**

Patient name:	**Date of birth:**
Patient postcode:	**NHS number:**
Name of referrer:	
Title/Position:	**Lab ID:**

Referrals will only be accepted from one of the following:	
Referrer	**Tick if this refers to you.**
Consultant Clinical Geneticist	
Consultant Cardiologist	

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:	
Criteria	**Tick if this patient meets criteria**
Index case with a prolonged QT interval on ECG **AND**	
A family history consistent with autosomal dominant inheritance **OR** a personal history of syncope without warning and/or aborted cardiac arrest.	

Additional Information:

For panel tests:
At risk family members where familial mutation is known do not require a full panel test but should be offered analysis of the known mutation

If the sample does not fulfil the clinical criteria or you are not one of the specified types of referrer and you still feel that testing should be performed please contact the laboratory to discuss testing of the sample.

Long QT syndrome 12 gene panel

NHS
UK Genetic Testing Network

Genes in panel test and associated conditions
Rows that are highlighted in yellow show where the gene is currently being fully analysed in the context of a single separate UKGTN test

HGNC standard name and symbol of the gene	HGNC number	OMIM number	OMIM standard name of condition and symbol	Mode of inheritance	OMIM number	Evidence of association between gene(s) and condition	% of horizontal coverage of gene	MLPA	Comments
AKAP9	HGNC:379	*604001	Long QT syndrome-11	AD	611820	PubMed: 18093912	100%	No	Not available in UKGTN
ANK2	HGNC:493	*106410	Cardiac arrhythmia, ankyrin-B-related Long QT syndrome-4	AD AD	600919 600919	PubMed: Various PubMed: Various	100%	No	Fully analysed as a single separate UKGTN test
CACNA1C	HGNC:1390	*114205	Brugada syndrome 3	AD	611875	PubMed: Various	100%	No	Not available in UKGTN
KCNE1	HGNC:6240	*176261	Long QT syndrome-5	AD	613695	PubMed: Various	100%	No	Available in UKGTN panel test
KCNE2	HGNC:6242	*603796	Atrial fibrillation, familial, 4 Long QT syndrome-6	AD AD	611493 613693	PubMed: Various PubMed: 10219239	100%	No	Available in UKGTN panel test
KCNH2	HGNC:6251	*152427	Long QT syndrome-2 Short QT syndrome-1	AD AD	613688 609620	PubMed: Various PubMed: Various	100%	No	Available in UKGTN panel test
KCNJ2	HGNC:6263	*600681	Atrial fibrillation, familial, 9 Short QT syndrome-3	AD AD	613980 609622	PubMed:15922306 PubMed:15761194	100%	No	Available in UKGTN panel test
KCNJ5	HGNC:6266	*600734	Long QT syndrome 13	AD	613485	PubMed: 20560207	100%	No	Not available in UKGTN
KCNQ1	HGNC:6294	*607542	Atrial fibrillation, familial, 3 Long QT syndrome-1 Short QT syndrome-2	AD AD/AR AD	607554 192500 609621	PubMed: 12522251 PubMed: Various PubMed: 15159330	100%	No	Available in UKGTN panel test
SCN4B	HGNC:10592	*608256	Long QT syndrome-10	AD	611819	PubMed: 17592081	100%	No	Not available in UKGTN
SCN5A	HGNC:10593	*600163	Atrial fibrillation, familial, 10 Brugada syndrome 1 Cardiomyopathy, dilated, 1E Long QT syndrome-3 Ventricular fibrillation, familial, 1	AD AD AD AD AD	614022 601144 601154 603830 603829	PubMed: Various PubMed: Various PubMed: Various PubMed: Various PubMed: 10940383	100%	No	Available in UKGTN panel test
SNTA1	HGNC:11167	*601017	Long QT syndrome 12	AD	612955	PubMed: 18591664	99%	No	Not available in UKGTN

Approval Date: Sept 2014

UKGTN Testing criteria

UK Genetic Testing Network

Disease(s): Catecholaminergic polymorphic ventricular tachycardia (CPVT)

Name of gene(s): RYR mutation analysis

Patient name: **Date of birth:**

Patient postcode: **NHS number:**

Name of referrer:

Title/Position:
Lab ID:

Referrals will only be accepted from one of the following:

Referrer	Tick if this refers to you.
Clinical Geneticists	
Cardiologists with a special interest in genetics	

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:

Criteria	Tick if this patient meets criteria
Family history of sudden unexplained death **AND**	
Absence of structural cardiac abnormalities **AND EITHER**	
Individuals with exercise-induced polymorphic ventricular arrhythmias **OR**	
Syncope occurring during physical activity or acute emotion	

If the sample does not fulfil the clinical criteria or you are not one of the specified types of referrer and you still feel that testing should be performed please contact the laboratory to discuss testing of the sample.

UKGTN Testing Criteria

Test name: **Molecular Autopsy (MolAut) 57 Gene Panel**	
Approved name and symbol of disorder/condition(s): See Appendix 1	**OMIM number(s):**
Approved name and symbol of gene(s): See Appendix 1	**OMIM number(s):**

Patient name:	**Date of birth:**
Patient postcode:	**NHS number:**
Name of referrer:	
Title/Position:	**Lab ID:**

Referrals will only be accepted from one of the following:	
Referrer	**Tick if this refers to you.**
Consultant Clinical Geneticist	
Consultant Cardiologist	

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:	
Criteria	**Tick if this patient meets criteria**
In samples available at autopsy from individuals (<40 years) with sudden unexplained death (normal morphology)	
In samples available at autopsy from individuals with sudden unexplained death (normal morphology) with family history of sudden cardiac death	

Additional Information:

For panel tests:
At risk family members where familial mutation is known do not require a full panel test but should be offered analysis of the known mutation

If the sample does not fulfil the clinical criteria or you are not one of the specified types of referrer and you still feel that testing should be performed please contact the laboratory to discuss testing of the sample

Molecular autopsy 57 gene panel

Genes in panel test and associated conditions
Rows that are highlighted in yellow show where the gene is currently being fully analysed in the context of a single separate UKGTN test

HGNC standard name and symbol of the gene	HGNC number	OMIM number	OMIM standard name of condition and symbol	Mode of inheritance	OMIM number	Evidence of association between gene(s) and condition	% of horizontal coverage of gene	MLPA	Comments
ABCC9	HGNC:60	*601439	Atrial fibrillation, familial, 12 Cardiomyopathy, dilated, 1O	AD AD	614050 608569	PubMed: Various PubMed: Various	100%	No	Fully analysed as a single separate UKGTN test
ACTC1	HGNC:143	*102540	Cardiomyopathy, dilated, 1R Cardiomyopathy, familial hypertrophic, 11	AD AD	613424 612098	PubMed: Various PubMed: Various	100%	No	Available in UKGTN panel test
ACTN2	HGNC:164	*102573	?Cardiomyopathy, dilated, 1AA	AD	612158	PubMed: 14567970	100%	No	Not available in UKGTN
AKAP9	HGNC:379	*604001	Long QT syndrome-11	AD	611820	PubMed: 18093912	100%	No	Not available in UKGTN
ANK2	HGNC:493	*106410	Cardiac arrhythmia, ankyrin-B-related Long QT syndrome-4	AD AD	600919 600919	PubMed: Various PubMed: Various	100%	No	Fully analysed as a single separate UKGTN test
CACNA1C	HGNC:1390	*114205	Brugada syndrome 3	AD	611875	PubMed: Various	100%	No	Not available in UKGTN
CALM1	HGNC:1442	*114180	Ventricular tachycardia, catecholaminergic polymorphic, 4	AD	614916	Pubmed Various	100%	No	Not available in UKGTN
CASQ2	HGNC:1513	*114251	Ventricular tachycardia, catecholaminergic polymorphic, 2	AR	611938	PubMed: Various	100%	No	Not available in UKGTN
CSRP3	HGNC:2472	*600824	Cardiomyopathy, dilated, 1M Cardiomyopathy, familial hypertrophic, 12	AD AD	607482 612124	PubMed: Various PubMed: Various	100%	No	Available in UKGTN panel test
DES	HGNC:2770	*125660	Cardiomyopathy, dilated, 1I	AD	604765	PubMed: Various	100%	No	Not available in UKGTN
DSC2	HGNC:3036	*125645	Arrhythmogenic right ventricular dysplasia 11	AD	610476	PubMed: Various	100%	Yes – selected exons	Fully analysed in a UKGTN panel test
DSG2	HGNC:3049	*125671	Arrhythmogenic right ventricular dysplasia 10 Cardiomyopathy, dilated, 1BB	AD AD	610193 610193	PubMed: Various PubMed: Various	100%	Yes – selected exons	Fully analysed in a UKGTN panel test

DSP	HGNC:3052	*125647	Arrhythmogenic right ventricular dysplasia 8	AD	607450	PubMed: Various	100%	Yes – selected exons	Fully analysed in a UKGTN panel test
EYA4	HGNC:3522	*603550	Cardiomyopathy, dilated, 1J	AD	605362	PubMed: 15735644	100%	No	Not available in UKGTN
FHL1	HGNC:3702	*300163	Emery-Dreifuss muscular dystrophy 6, X-linked	XLR	300696	Cardiomyopathy described: PubMed: 18179888	100%	No	Available in UKGTN panel test
GPD1L	HGNC:28956	*611778	Brugada syndrome 2	AD	611777	PubMed: Various	100%	No	Not available in UKGTN
GLA	HGNC:4296	*300644	Fabry disease, cardiac variant	XLR	300644	PubMed: Various	100%	No	Available in UKGTN panel test
HCN4	HGNC:16882	*605206	Brugada syndrome 8	AD	613123	PubMed: 19165230	100%	No	Not available in UKGTN
JUP	HGNC:6207	*173325	Arrhythmogenic right ventricular dysplasia 12	AD	611528	PubMed: 17924338	100%	Yes – selected exons	Not available in UKGTN
KCNE1	HGNC:6240	*176261	Long QT syndrome-5	AD	613695	PubMed: Various	100%	No	Available in UKGTN panel test
KCNE2	HGNC:6242	*603796	Atrial fibrillation, familial, 4 Long QT syndrome-6	AD AD	611493 613693	PubMed: Various PubMed: 10219239	100%	No	Available in UKGTN panel test
KCNE3	HGNC:6243	*604433	Brugada syndrome 6	AD	613119	PubMed: Various	100%	No	Not available in UKGTN
KCNH2	HGNC:6251	*152427	Long QT syndrome-2 Short QT syndrome-1	AD AD	613688 609620	PubMed: Various PubMed: Various	100%	No	Available in UKGTN panel test
KCNJ2	HGNC:6263	*600681	Atrial fibrillation, familial, 9 Short QT syndrome-3	AD AD	613980 609622	PubMed:15922306 PubMed:15761194	100%	No	Available in UKGTN panel test
KCNJ5	HGNC:6266	*600734	Long QT syndrome 13	AD	613485	PubMed: 20560207	100%	No	Not available in UKGTN
KCNQ1	HGNC:6294	*607542	Atrial fibrillation, familial, 3 Long QT syndrome-1 Short QT syndrome-2	AD AD/AR AD	607554 192500 609621	PubMed: 12522251 PubMed: Various PubMed: 15159330	100%	No	Available in UKGTN panel test
LAMP2	HGNC:6501	*309060	Danon disease	AD	300257	PubMed: Various	100%	No	Available in UKGTN panel test
LMNA	HGNC:6636	*150330	Cardiomyopathy, dilated, 1A	AD	115200	Pubmed Various	100%	No	Available in UKGTN Panel

Molecular autopsy 57 gene panel

MYBPC3	HGNC:7551	*600958	Cardiomyopathy, dilated, 1MM Cardiomyopathy, familial hypertrophic, 4 Left ventricular noncompaction 10	AD AD AD	615396 115197 615396	PubMed: Various PubMed: Various PubMed: 21551322	100%	No	Available in UKGTN panel test
MYH6	HGNC:7576	*160710	Atrial septal defect 3 Cardiomyopathy, dilated, 1EE Cardiomyopathy, familial hypertrophic, 14	AD AD AD	614089 613252 613251	PubMed: Various PubMed: Various PubMed: Various	100%	No	Not available in UKGTN
MYH7	HGNC:7577	*160760	Cardiomyopathy, dilated, 1S Cardiomyopathy, familial hypertrophic, 1	AD AD	613426 192600	PubMed: Various PubMed: Various	100%	No	Available in UKGTN panel test
MYL2	HGNC:7583	*160781	Cardiomyopathy, familial hypertrophic, 10	AD	608758	PubMed: Various	100%	No	Available in UKGTN panel test
MYL3	HGNC:7584	*160790	Cardiomyopathy, familial hypertrophic, 8	AD/AR	608751	PubMed: Various	100%	No	Available in UKGTN panel test
MYLK2	HGNC:16243	*606566	Cardiomyopathy, hypertrophic, midventricular, digenic	Digenic	192600	PubMed: 11733062	100%	No	Not available in UKGTN
NEXN	HGNC:29557	*613121	Cardiomyopathy, dilated, 1CC Cardiomyopathy, familial hypertrophic, 20	AD AD	613122 613876	PubMed: Various PubMed: Various	100%	No	Not available in UKGTN
PKP2	HGNC:9024	*602861	Arrhythmogenic right ventricular dysplasia 9	AD	609040	[PubMed: 15489853	100%	Yes – selected exons	Fully analysed in a UKGTN panel test
PLN	HGNC:9080	*172405	Cardiomyopathy, dilated, 1P Cardiomyopathy, familial hypertrophic, 18	AD AD	609909 613874	PubMed: Various PubMed: Various	100%	No	Available in UKGTN panel test
PRKAG2	HGNC:9386	*602743	Cardiomyopathy, familial hypertrophic 6	AD	600858	PubMed: Various	100%	No	Available in UKGTN panel test
RBM20	HGNC:27424	*613171	Cardiomyopathy, dilated, 1DD	AD	613172	PubMed: Various	100%	No	Not available in UKGTN
RYR2	HGNC:10484	*180902	Arrhythmogenic right ventricular dysplasia 2 Ventricular tachycardia, catecholaminergic polymorphic, 1	AD AD	600996 604772	PubMed: Various PubMed: 11159936	100%	Yes – selected exons	Fully analysed in the context of a single separate UKGTN test

SCN1B	HGNC:10586	*600235	Atrial fibrillation, familial, 13 Brugada syndrome 5	AD AD	615377 612838	PubMed: Various PubMed: 18464934	98%	No	Not available in UKGTN
SCN3B	HGNC:20665	*608214	Brugada syndrome 7	AD	613120	PubMed: 20031595	100%	No	Not available in UKGTN
SCN4B	HGNC:10592	*608256	Long QT syndrome-10	AD	611819	PubMed: 17592081	100%	No	Not available in UKGTN
SCN5A	HGNC:10593	*600163	Atrial fibrillation, familial, 10 Brugada syndrome 1 Cardiomyopathy, dilated, 1E Long QT syndrome-3 Ventricular fibrillation, familial, 1	AD AD AD AD AD	614022 601144 601154 603830 603829	PubMed: Various PubMed: Various PubMed: Various PubMed: Various PubMed: 10940383	100%	No	Available in UKGTN panel test
SGCD	HGNC:10807	*601411	Cardiomyopathy, dilated, 1L	AD	606685	PubMed: Various	100%	No	Not available in UKGTN
SLC25A4	HGNC:10990	*103220	Mitochondrial DNA depletion syndrome 12 (cardiomyopathic type)	AR	615418	PubMed: 22187496	100%	No	Available in UKGTN panel test
SNTA1	HGNC:11167	*601017	Long QT syndrome 12	AD	612955	PubMed: 18591664	99%	No	Not available in UKGTN
TCAP	HGNC:11610	*604488	Cardiomyopathy, dilated, 1N	AD	607487	PubMed: 12507422	100%	No	Not available in UKGTN
TMEM43	HGNC:28472	*612048	Arrhythmogenic right ventricular dysplasia 5	AD	604400	PubMed: Various	100%	No	Not available in UKGTN
TMPO	HGNC:11875	*188380	Cardiomyopathy, dilated, 1T	AD	613740	PubMed: 16247757	100%	No	Not available in UKGTN
TNNC1	HGNC:11943	*191040	Cardiomyopathy, dilated, 1Z Cardiomyopathy, familial hypertrophic, 13	AD AD	611879 613243	PubMed: 15542288 PubMed: Various	100%	No	Not available in UKGTN
TNNI3	HGNC:11947	*191044	Cardiomyopathy, dilated, 1FF Cardiomyopathy, dilated, 2A Cardiomyopathy, familial hypertrophic, 7 Cardiomyopathy, familial restrictive	AD AR AD AD	613286 611880 613690 115210	PubMed: Various PubMed: 15070570 PubMed: Various PubMed: Various	100%	No	Available in UKGTN panel test

Molecular autopsy 57 gene panel

TNNT2	HGNC:11949	*191045	Cardiomyopathy, dilated, 1D Cardiomyopathy, familial hypertrophic, 2 Cardiomyopathy, familial restrictive, 3	AD AD AD	601494 115195 612422	PubMed: Various PubMed: Various PubMed: 16651346	100%	No	Available in UKGTN panel test
TPM1	HGNC:12010	*191010	Cardiomyopathy, dilated, 1Y Cardiomyopathy, familial hypertrophic, 3	AD AD	611878 115196	PubMed: Various PubMed: 11273725	100%	No	Available in UKGTN panel test
TRDN	HGNC:12261	*603283	Ventricular tachycardia, catecholaminergic polymorphic, 5, with or without muscle weakness	AR	615441	Pubmed Various	100%	No	Not available in UKGTN
TTN	HGNC:12403	*188840	Cardiomyopathy, dilated, 1G Cardiomyopathy, familial hypertrophic, 9	AD AD	604145 613765	PubMed: Various PubMed: Various	100%	No	Not available in UKGTN
VCL	HGNC:12665	*193065	Cardiomyopathy, dilated, 1W Cardiomyopathy, familial hypertrophic, 15	AD AD	611407 613255	PubMed: 11815424 PubMed: 16712796	100%	No	Not available in UKGTN

UKGTN Testing Criteria

Test name: **Hypertrophic Cardiomyopathy (HCM) 22 Gene Panel**	
Approved name and symbol of disorder/condition(s): See Appendix 1	**OMIM number(s):**
Approved name and symbol of gene(s): See Appendix 1	**OMIM number(s):**

Patient name:	**Date of birth:**
Patient postcode:	**NHS number:**
Name of referrer:	
Title/Position:	**Lab ID:**

Referrals will only be accepted from one of the following:	
Referrer	**Tick if this refers to you.**
Consultant Clinical Geneticist	
Consultant Cardiologist	

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:	
Criteria	**Tick if this patient meets criteria**
Unexplained left ventricular hypertrophy on cardiac imaging (e.g. echocardiogram or magnetic resonance)	

Additional Information:

For panel tests:
At risk family members where familial mutation is known do not require a full panel test but should be offered analysis of the known mutation

If the sample does not fulfil the clinical criteria or you are not one of the specified types of referrer and you still feel that testing should be performed please contact the laboratory to discuss testing of the sample.

Hypertrophic cardiomyopathy 22 gene panel

Genes in panel test and associated conditions. Rows that are highlighted in yellow show where the gene is currently being fully analysed in the context of a single separate UKGTN test

HGNC standard name and symbol of the gene	HGNC number	OMIM number	OMIM standard name of condition and symbol	Mode of inheritance	OMIM number	Evidence of association between gene(s) and condition	% of horizontal coverage of gene	MLPA	Comments
ACTC1	HGNC:143	*102540	Cardiomyopathy, dilated, 1R Cardiomyopathy, familial hypertrophic, 11	AD AD	613424 612098	PubMed: Various PubMed: Various	100%	No	Available in UKGTN panel test
CSRP3	HGNC:2472	*600824	Cardiomyopathy, dilated, 1M Cardiomyopathy, familial hypertrophic, 12	AD AD	607482 612124	PubMed: Various PubMed: Various	100%	No	Available in UKGTN panel test
FHL1	HGNC:3702	*300163	Emery-Dreifuss muscular dystrophy 6, X-linked	XLR	300696	Cardiomyopathy described: PubMed: 18179888	100%	No	Available in UKGTN panel test
GLA	HGNC:4296	*300644	Fabry disease, cardiac variant	XLR	300644	PubMed: Various	100%	No	Available in UKGTN panel test
LAMP2	HGNC:6501	*309060	Danon disease	AD	300257	PubMed: Various	100%	No	Available in UKGTN panel test
MYBPC3	HGNC:7551	*600958	Cardiomyopathy, dilated, 1MM Cardiomyopathy, familial hypertrophic, 4 Left ventricular noncompaction 10	AD AD AD	615396 115197 615396	PubMed: Various PubMed: Various PubMed: 21551322	100%	No	Available in UKGTN panel test
MYH6	HGNC:7576	*160710	Atrial septal defect 3 Cardiomyopathy, dilated, 1EE Cardiomyopathy, familial hypertrophic, 14	AD AD AD	614089 613252 613251	PubMed: Various PubMed: Various PubMed: Various	100%	No	Not available in UKGTN
MYH7	HGNC:7577	*160760	Cardiomyopathy, dilated, 1S Cardiomyopathy, familial hypertrophic, 1	AD AD	613426 192600	PubMed: Various PubMed: Various	100%	No	Available in UKGTN panel test
MYL2	HGNC:7583	*160781	Cardiomyopathy, familial hypertrophic, 10	AD	608758	PubMed: Various	100%	No	Available in UKGTN panel test
MYL3	HGNC:7584	*160790	Cardiomyopathy, familial hypertrophic, 8	AD/AR	608751	PubMed: Various	100%	No	Available in UKGTN panel test
MYLK2	HGNC:16243	*606566	Cardiomyopathy, hypertrophic, midventricular, digenic	Digenic	192600	PubMed: 11733062	100%	No	Not available in UKGTN
NEXN	HGNC:29557	*613121	Cardiomyopathy, dilated, 1CC	AD	613122	PubMed: Various	100%	No	Not available in UKGTN

			Cardiomyopathy, familial hypertrophic, 20	AD	613876	PubMed: Various			
PLN	HGNC:9080	*172405	Cardiomyopathy, dilated, 1P Cardiomyopathy, familial hypertrophic, 18	AD AD	609909 613874	PubMed: Various PubMed: Various	100%	No	Available in UKGTN panel test
PRKAG2	HGNC:9386	*602743	Cardiomyopathy, familial hypertrophic 6	AD	600858	PubMed: Various	100%	No	Available in UKGTN panel test
SLC25A4	HGNC:10990	*103220	Mitochondrial DNA depletion syndrome 12 (cardiomyopathic type)	AR	615418	PubMed: 22187496	100%	No	Available in UKGTN panel test
TCAP	HGNC:11610	*604488	Cardiomyopathy, dilated, 1N	AD	607487	PubMed: 12507422	100%	No	Not available in UKGTN
TNNC1	HGNC:11943	*191040	Cardiomyopathy, dilated, 1Z Cardiomyopathy, familial hypertrophic, 13	AD AD	611879 613243	PubMed: 15542288 PubMed: Various	100%	No	Not available in UKGTN
TNNI3	HGNC:11947	*191044	Cardiomyopathy, dilated, 1FF Cardiomyopathy, dilated, 2A Cardiomyopathy, familial hypertrophic, 7 Cardiomyopathy, familial restrictive	AD AR AD AD	613286 611880 613690 115210	PubMed: Various PubMed: 15070570 PubMed: Various PubMed: Various	100%	No	Available in UKGTN panel test
TNNT2	HGNC:11949	*191045	Cardiomyopathy, dilated, 1D Cardiomyopathy, familial hypertrophic, 2 Cardiomyopathy, familial restrictive, 3	AD AD AD	601494 115195 612422	PubMed: Various PubMed: Various PubMed: 16651346	100%	No	Available in UKGTN panel test
TPM1	HGNC:12010	*191010	Cardiomyopathy, dilated, 1Y Cardiomyopathy, familial hypertrophic, 3	AD AD	611878 115196	PubMed: Various PubMed: 11273725	100%	No	Available in UKGTN panel test
TTN	HGNC:12403	*188840	Cardiomyopathy, dilated, 1G Cardiomyopathy, familial hypertrophic, 9	AD AD	604145 613765	PubMed: Various PubMed: Various	100%	No	Not available in UKGTN
VCL	HGNC:12665	*193065	Cardiomyopathy, dilated, 1W Cardiomyopathy, familial hypertrophic, 15	AD AD	611407 613255	PubMed: 11815424 PubMed: 16712796	100%	No	Not available in UKGTN

Approval Date: Sept 2014

Appendix 2

UKGTN testing criteria for **thoracic aortic aneurysm syndromes**

1. Thoracic aortic aneurysm 9 gene panel
2. Aortopathy 17 gene panel
3. Ehlers-Danlos syndrome 12 gene panel
4. Ehlers-Danlos syndrome Type I, Type II
5. Ehlers-Danlos syndrome Type VI
6. Familial thoracic aortic aneurysms and dissection (TAAD)
7. Loeys-Dietz syndrome Type 1A, 1B, 2A, 2B
8. Loeys-Dietz syndrome Type3, Type 4
9. Marfan syndrome
10. Marfan syndrome Type 1 *FBN1* negative

UKGTN Testing Criteria

Test name: **Thoracic Aortic Aneurysm (AA) 9 Gene Panel**	
Approved name and symbol of disorder/condition(s): See Appendix 1	**OMIM number(s):**
Approved name and symbol of gene(s): See Appendix 1	**OMIM number(s):**

Patient name:	**Date of birth:**
Patient postcode:	**NHS number:**
Name of referrer:	
Title/Position:	**Lab ID:**

Referrals will only be accepted from one of the following:	
Referrer	**Tick if this refers to you.**
Consultant Clinical Geneticist	
Consultant Cardiologist	

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:	
Criteria	**Tick if this patient meets criteria**
Dilation and/or dissection of the ascending thoracic aorta, **OR** dissection of the descending aorta just distal to the subclavian artery	
AND Family history of TAAD or unusually early presentation of dissection	

Additional Information:

For panel tests:
At risk family members where familial mutation is known do not require a full panel test but should be offered analysis of the known mutation

If the sample does not fulfil the clinical criteria or you are not one of the specified types of referrer and you still feel that testing should be performed please contact the laboratory to discuss testing of the sample.

Thoracic aortic aneurysm 9 gene panel

Genes in panel test and associated conditions
Rows that are highlighted in yellow show where the gene is currently being fully analysed in the context of a single separate UKGTN test

HGNC standard name and symbol of the gene	HGNC number	OMIM number	OMIM standard name of condition and symbol	Mode of inheritance	OMIM number	Evidence of association between gene(s) and condition	% of horizontal coverage of gene	MLPA	Comments
ACTA2	HGNC:130	*102620	Aortic aneurysm, familial thoracic 6 Moyamoya disease 5 Multisystemic smooth muscle dysfunction syndrome	AD AD AD	611788 614042 613834	PubMed: Various PubMed: Various PubMed: Various	100%	No	Fully analysed as a single separate UKGTN test
COL3A1	HGNC:2201	*120180	Ehlers-Danlos syndrome, type III Ehlers-Danlos syndrome, type IV	AD AD	130020 130050	PubMed: 7833919 PubMed: Various	100%	No	Fully analysed as a single separate UKGTN test
FBN1	HGNC:3603	*134797	Aortic aneurysm, ascending, and dissection	AD	No phenotype MIM number	PubMed: Various	100%	No	Fully analysed as a single separate UKGTN test
KCNN1	HGNC:6290	*602982	Ventricular tachyarrhythmias	AD	No OMIM reference	Pubmed: 23086994	100%	No	Not available in UKGTN
MYH11	HGNC:7569	*160745	Aortic aneurysm, familial thoracic 4	AD	132900	PubMed: Various	100%	No	Available in UKGTN panel test
SMAD3	HGNC:6769	*603109	Loeys-Dietz syndrome, type 3	AD	613795	PubMed: Various	100%	No	Available in UKGTN panel test
TGFB2	HGNC:11768	*190220	Loeys-Dietz syndrome, type 4	AD	614816	PubMed: Various	100%	No	Available in UKGTN panel test
TGFBR1	HGNC:11772	*190181	Loeys-Dietz syndrome, type 1A Loeys-Dietz syndrome, type 2A	AD AD	609192 608967	PubMed: Various PubMed: Various	92.5%	No	Fully analysed in a UKGTN panel test
TGFBR2	HGNC:11773	*190182	Loeys-Dietz syndrome, type 1B Loeys-Dietz syndrome, type 2B	AD AD	610168 610380	PubMed: Various PubMed: Various	100%	No	Fully analysed in a UKGTN panel test

UKGTN Testing Criteria

Test name: **Aortopathy 17 Gene Panel**	
Approved name and symbol of disorder/condition(s): See appendix 1	**OMIM number(s):**
Approved name and symbol of gene(s): See appendix 1	**OMIM number(s):**

Patient name:	**Date of birth:**
Patient postcode:	**NHS number:**
Name of referrer:	
Title/Position:	**Lab ID:**

Referrals will only be accepted from one of the following:	
Referrer	**Tick if this refers to you.**
Consultant Clinical Geneticist	
Consultant Adult/Paediatric Cardiologist (in liaison with a Clinical Geneticist)	

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:	
Criteria	**Tick if this patient meets criteria**
Affected individual with a non-specific phenotype and a strong clinical suspicion of a monogenic predisposition to aortopathy, with or without a family history.	
OR Diagnostic testing for Marfan syndrome, Ehlers Danlos syndrome, or Loeys Dietz syndrome has not identifed a causative mutation, and high clinical suspicion of condition predisposing to aortic/arterial disease	

Additional Information:
For panel tests:
At risk family members where familial mutation is known do not require a full panel test but should be offered analysis of the known mutation

If the sample does not fulfil the clinical criteria or you are not one of the specified types of referrer and you still feel that testing should be performed please contact the laboratory to discuss testing of the sample.

Aortopathy 17 gene panel

Genes in panel test and associated conditions

HGNC standard name and symbol of the gene	HGNC number	OMIM number	OMIM standard name of condition and symbol	Mode of inheritance	OMIM number	Evidence of association between gene(s) and condition	% of horizontal coverage of gene	MLPA	Comments
Transforming Growth Factor Beta receptor 1; TGFBR1	11772	190181	Loeys-Dietz syndrome, type 1; LDS1	AD	609192	Loeys, B. L., et al. Aneurysm syndromes caused by mutations in the TGF-beta receptor. New Eng. J. Med. 355: 788-798, 2006.	93.60%	N	Existing provider
Transforming Growth Factor Beta receptor 2; TGFBR2	11773	190182	Loeys-Dietz syndrome, type 2; LDS2	AD	610168	Loeys, B. L., et al. Aneurysm syndromes caused by mutations in the TGF-beta receptor. New Eng. J. Med. 355: 788-798, 2006.	95.20%	N	Existing provider
SMAD family member 3; SMAD3	6769	603109	Loeys-Dietz syndrome, type 3; LDS3	AD	613795	van de Laar, et al. Phenotypic spectrum of the SMAD3-related aneurysms-osteoarthritis syndrome. J. Med. Genet. 49: 47-57, 2012.	100%	N	Existing provider
Transforming growth factor, beta 2; TGFB2	11768	190220	Loeys-Dietz syndrome, type 4; LDS4	AD	614816	Boileau, C., et al. TGFB2 mutations cause familial thoracic aortic aneurysms and dissections associated with mild systemic features of Marfan syndrome. Nature Genet. 44: 916-921, 2012.	100%	N	Existing provider
Collagen, Type 3, Alpha-1; COL3A1	2201	120180	Ehlers-Danlos syndrome, type 4, autosomal dominant; EDS4	AD	130050	Superti-Furga, A., Steinmann, B. Impaired secretion of type III procollagen in Ehlers-Danlos syndrome type IV fibroblasts: correction of the defect by incubation at reduced temperature and demonstration of subtle alterations in the triple-helical region of the molecule. Biochem. Biophys. Res. Commun. 150: 140-147, 1988	100%	N	Existing provider
Fibrillin; FBN1	3603	134797	Marfan syndrome; MFS	AD	154700	Hayward, C., et al. Fibrillin (FBN1) mutations in Marfan syndrome. (Letter) Hum. Mutat. 1: 79, 1992.	100%	N	Existing provider

Actin, Alpha-2, Smooth Muscle, Aorta; ACTA2;	130	102620	Aortic aneurysm, familial thoracic 6; AAT6	AD	611788	Guo, D.-C., et al. Mutations in smooth muscle alpha-actin (ACTA2) lead to thoracic aortic aneurysms and dissections. Nature Genet. 39: 1488-1493, 2007.	100%	N	Existing provider
Myosin, heavy chain 11, smooth muscle; MYH11	7569	160745	Aortic aneurysm, familial thoracic 4; AAT4	AD	132900	Zhu, L., et al. Mutations in myosin heavy chain 11 cause a syndrome associating thoracic aortic aneurysm/aortic dissection and patent ductus arteriosus. Nature Genet. 38: 343-349, 2006.	100%	N	Existing provider
Myosin light chain kinase; MYLK	7590	600922	Aortic aneurysm, familial thoracic 7; AAT7	AD	613780	Wang, L., et al. Mutations in myosin light chain kinase cause familial aortic dissections. Am. J. Hum. Genet. 87: 701-707, 2010.	98%	N	
Solute carrier family 2, member 10; SLC2A10	13444	606145	Arterial tortuosity syndrome; ATS	AR	208050	Coucke, P. J., et al. Mutations in the facilitative glucose transporter GLUT10 alter angiogenesis and cause arterial tortuosity syndrome. Nature Genet. 38: 452-457, 2006.	100%	N	
NOTCH, Drosophila, Homolog of, 1; NOTCH1	7881	190198	Aortic valve disease,1; AOVD1	AD	109730	Garg, V., et al. Mutations in NOTCH1 cause aortic valve disease. Nature 437: 270-274, 2005.	98%	N	
Fibrillin 2; FBN2	3604	612570	Contractural arachnodactyly, congenital; CCA	AD	121050	Putnam, E. A., et al. Fibrillin-2 (FBN2) mutations result in the Marfan-like disorder, congenital contractural arachnodactyly. Nature Genet. 11: 456-458, 1995.	99.90%	N	
Filamin A; FLNA	3754	300017	Cardiac valvular dysplasia, X-linked; CVD1	XLD	314400	Kyndt, F., Gueffet, J.-P., Probst, V., Jaafar, P., Legendre, A., Le Bouffant, F., Toquet, C., Roy, E., McGregor, L., Lynch, S. A., Newbury-Ecob, R., Tran, V., Young, I., Trochu, J.-N., Le Marec, H., Schott, J.-J. Mutations in the gene encoding filamin A as a cause for familial cardiac valvular dystrophy. Circulation 115: 40-49, 2007	99.70%	N	existing provider

Aortopathy 17 gene panel

V-SKI AVIAN SARCOMA VIRAL ONCOGENE HOMOLOG; SKI	10896	164780	SHPRINTZEN-GOLDBERG CRANIOSYNOSTOSIS SYNDROME; SGS	AD	182212	Doyle, A. J., Doyle, J. J., Bessling, S. L., Maragh, S., Lindsay, M. E., Schepers, D., Gillis, E., Mortier, G., Homfray, T., Sauls, K., Norris, R. A., Huso, N. D., and 22 others. Mutations in the TGF-beta repressor SKI cause Shprintzen-Goldberg syndrome with aortic aneurysm. Nature Genet. 44: 1249-1254, 2012.	100%	N	
SMAD family member 4; SMAD4	6770	600993	JUVENILE POLYPOSIS/HEREDITARY HEMORRHAGIC TELANGIECTASIA SYNDROME; JPHT	AD	175050	Gallione, C. J., Richards, J. A., Letteboer, T. G. W., Rushlow, D., Prigoda, N. L., Leedom, T. P., Ganguly, A., Castells, A., Ploos van Amstel, J. K., Westermann, C. J. J., Pyeritz, R. E., Marchuk, D. A. SMAD4 mutations found in unselected HHT patients. J. Med. Genet. 43: 793-797, 2006.	78.70%	N	existing provider
transforming growth factor, beta 3; TGFB3	11769	190230	ARRHYTHMOGENIC RIGHT VENTRICULAR DYSPLASIA, FAMILIAL, 1; ARVD1	AD	107970	Beffagna, G., Occhi, G., Nava, A., Vitiello, L., Ditadi, A., Basso, C., Bauce, B., Carraro, G., Thiene, G., Towbin, J. A., Danieli, G. A., Rampazzo, A. Regulatory mutations in transforming growth factor-beta-3 gene cause arrhythmogenic right ventricular cardiomyopathy type 1. Cardiovasc. Res. 65: 366-373, 2005.	99%	N	
EGF containing fibulin-like extracellular matrix protein 2; EFEMP2	3219	604633	CUTIS LAXA, AUTOSOMAL RECESSIVE, TYPE IB; ARCL1B	AR	614437	Hucthagowder, V., Sausgruber, N., Kim, K. H., Angle, B., Marmorstein, L. Y., Urban, Z. Fibulin-4: a novel gene for an autosomal recessive cutis laxa syndrome. Am. J. Hum. Genet. 78: 1075-1080, 2006.	100%	N	

UKGTN Testing Criteria

Test name: **Ehlers-Danlos 12 gene panel**	
Approved name and symbol of disorder/condition(s): See appendix 1	**OMIM number(s):**
Approved name and symbol of gene(s): See appendix 1	**OMIM number(s):**

Patient name:	**Date of birth:**
Patient postcode:	**NHS number:**
Name of referrer:	
Title/Position:	**Lab ID:**

Referrals will only be accepted from one of the following:	
Referrer	**Tick if this refers to you.**
Consultant Clinical Geneticist	
Consultant Paediatrician	
Consultant Neurologist	
Consultant Dermatologist	

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:	
Criteria	**Tick if this patient meets criteria**
Skin hyperextensibility **AND** Joint hypermobility and laxity **AND** one of the following:	
Widening atrophic scars (tissue fragility)	
Easy bruising	
Muscle hypotonia	
Scoliosis	
Scleral fragility	

Additional Information:
At risk family members where familial mutation is known do not require a full panel test but should be offered analysis of the known mutation

If the sample does not fulfil the clinical criteria or you are not one of the specified types of referrer and you still feel that testing should be performed please contact

Approval Date: Sept 2014

Ehlers-Danlos syndrome 12 gene panel

NHS
UK Genetic Testing Network

Genes in panel test and associated conditions
Rows that are highlighted in yellow show where the gene is currently being fully analysed in the context of a single separate UKGTN test

HGNC standard name and symbol of the gene	HGNC number	OMIM number	OMIM standard name of condition and symbol	Mode of inheritance	OMIM number	Evidence of association between gene(s) and condition	% of horizontal coverage of gene	MLPA	Comments
collagen, type III, alpha 1; COL3A1	2201	120180	EHLERS-DANLOS SYNDROME, TYPE IV, AUTOSOMAL DOMINANT	AD	130050	https://eds.gene.le.ac.uk/home.php?select_db=COL3A1 ≥ 342 unique variants in 476 individuals	100	YES	EDS/FTAA
collagen, type V, alpha 1; COL5A1	2209	120215	EHLERS-DANLOS SYNDROME, TYPE I; EHLERS-DANLOS SYNDROME, TYPE II	AD	130000; 130010	https://eds.gene.le.ac.uk/home.php?select_db=COL5A1 ≥ 165 unique variants detected in 180 individuals	100	YES	EDS
collagen, type V, alpha 2; COL5A2	2210	120190	EHLERS-DANLOS SYNDROME, TYPE I;	AD	130000	https://eds.gene.le.ac.uk/home.php?select_db=COL5A2 ≥ 46 unique variants detected in 45 individuals	100	NO	EDS
carbohydrate (N-acetylgalactosamine 4-0) sulfotransferase 14; CHST14	24464	608429	EHLERS-DANLOS SYNDROME, MUSCULOCONTRACTURAL TYPE 1; EDSMC1	AR	601776	https://eds.gene.le.ac.uk/home.php?select_db=CHST14 ≥ 12 unique variants detected in 17 individuals	100	NO	EDS
procollagen-lysine, 2-oxoglutarate 5-dioxygenase 1; PLOD1	9081	153454	EHLERS-DANLOS SYNDROME, TYPE VI; EDS6	AR	225400	https://eds.gene.le.ac.uk/home.php?select_db=PLOD1 ≥38 unique variants detected in 65 individuals	100	YES	EDS
FK506 binding protein 14, 22 kDa; FKBP14	18625	614505	EHLERS-DANLOS SYNDROME WITH PROGRESSIVE KYPHOSCOLIOSIS, MYOPATHY, AND HEARING LOSS; EDSKMH	AR	614557	Baumann et al. (2012) Am. J. Hum. Genet. 90: 201-216 - 2 unique variants detected in 6 individuals	100	NO	EDS
Ras and Rab interactor 2; RIN2	18750	610222	MACROCEPHALY, ALOPECIA, CUTIS LAXA, AND SCOLIOSIS	AR	613075	Basel-Vanagaite et al. (2009) Am. J. Hum. Genet. 85: 254-263 - 1 unique variant in 3 individuals ; Syx et al. (2010) Hum. Genet. 128: 79-88 - 1 unique variant in 3 individuals	100	NO	EDS/Cutis Laxa
PR domain containing 5; PRDM5	9349	614161	BRITTLE CORNEA SYNDROME 2; BCS2	AR	614170	Aldahmesh et al. (2012) Clin. Genet. 81: 198-199 - 1 unique variant in 1 individual Burkitt Wright et al. (2011) Am. J. Hum. Genet. 88: 767-777 - 2 unique variants in 14 individuals	100	NO	EDS

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zinc finger protein 469; ZNF469	23216	612078	BRITTLE CORNEA SYNDROME 1; BCS1	AR	229200	Abu et al. (2008) Am. J. Hum. Genet. 82: 1217-1222 - 2 unique variants detected in 5 individuals Christensen et al. (2010) Invest. Ophthal. Vis. Sci. 51: 47-52 - 1 unique variant detected in 2 individuals; Khan et al. (2010) Arch. Ophthal. 128: 1376-1379 - 1 unique variants detected in consanguineous family	100	NO	EDS
xylosylprotein beta 1,4-galactosyltransferase, polypeptide 7 ; B4GALT7	930	604327	EHLERS-DANLOS SYNDROME, PROGEROID TYPE, 1; EDSP1	AR	130070	https://eds.gene.le.ac.uk/home.php?select_db=B4GALT7 ≥ 3 unique variants detected in 2 individuals	100	NO	EDS
solute carrier family 39 (zinc transporter), member 13; SLC39A13	20859	608735	SPONDYLOCHEIRODYSPLASIA, EHLERS-DANLOS SYNDROME-LIKE	AR	612350	Giunta et al. (2008) Am. J. Hum. Genet. 82: 1290-1305 - 1 unique variants detected in 6 individuals Fukada et al. (2008) PLoS One 3: e3642 - 1 unique variants detected in 2 individuals	100	NO	EDS
ADAM metallopeptidase with thrombospondin type 1 motif, 2; ADAMTS2	218	604539	EHLERS-DANLOS SYNDROME, TYPE VII, AUTOSOMAL RECESSIVE	AR	225410	https://eds.gene.le.ac.uk/home.php?select_db=ADAMTS2 ≥ 20 variants detected	100	NO	EDS

UKGTN Testing criteria

Name of Disease(s):
EHLERS-DANLOS SYNDROME, TYPE I (130000)
EHLERS-DANLOS SYNDROME, TYPE II (130010)

Name of gene(s):
collagen, type V, alpha 1; COL5A1 (120215)
collagen, type V, alpha 2; COL5A2 (120190)

Patient name: **Date of birth:**

Patient postcode: **NHS number:**

Name of referrer:

Title/Position:
Lab ID:

Referrals will only be accepted from one of the following:

Referrer	Tick if this refers to you.
Consultant Clinical Geneticist	
Consultant Dermatologist	
Consultant Rheumatologist	

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:

Criteria	Tick if this patient meets criteria
Skin Hyperextensibility **AND**	
Widening atrophic scars(tissue fragility) **AND**	
Joint Hypermobility	

If the sample does not fulfil the clinical criteria or you are not one of the specified types of referrer and you still feel that testing should be performed please contact the laboratory to discuss testing of the sample.

UKGTN Testing criteria

Name of Disease(s): EHLERS-DANLOS SYNDROME, TYPE VI (225400)

Name of gene(s): procollagen-lysine 1, 2-oxoglutarate 5-dioxygenase 1; PLOD1 (153454)

Patient name: **Date of birth:**

Patient postcode: **NHS number:**

Name of referrer:

Title/Position:
Lab ID:

Referrals will only be accepted from one of the following:

Referrer	Tick if this refers to you.
Clinical Geneticist	

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:

Criteria	Tick if this patient meets criteria
At least 3 of 4 below:	
1. Severe muscular hypotonia at birth	
2. Generalised joint laxity/recurrent joint dislocations	
3. Kyphoscoliosis at birth, which is progressive	
4. Scleral fragility and rupture of the ocular globe with high myopia	

If the sample does not fulfil the clinical criteria or you are not one of the specified types of referrer and you still feel that testing should be performed please contact the laboratory to discuss testing of the sample.

UKGTN Testing Criteria

Approved name and symbol of disease/condition(s): Familial Thoracic Aortic Aneurysms Dissections (TAAD)	**OMIM number(s):** 132900, 611788,
Approved name and symbol of gene(s): *MYH11, ACTA2, TGFBR1, TGFRB2*	**OMIM number(s):** 160745, 102620, 190181, 190182

Patient name:	**Date of birth:**
Patient postcode:	**NHS number:**
Name of referrer:	
Title/Position:	**Lab ID:**

Referrals will only be accepted from one of the following:	
Referrer	**Tick if this refers to you.**
Consultant Clinical Geneticist	
Consultant Cardiologist in liaison with clinical geneticist	

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:	
Criteria	**Tick if this patient meets criteria**
Dilation and/or dissection of the ascending thoracic aorta, **OR** dissection of the descending aorta just distal to the subclavian artery	
AND 2. Family history of TAAD or unusually early presentation of dissection	
AND exclusion of Marfan syndrome, Loeys-Dietz aortic syndrome and other connective tissue abnormalities	
OR Family history of known mutation in ACTA2/MYH11/TGFBR1/TGFBR2	

If the sample does not fulfil the clinical criteria or you are not one of the specified types of referrer and you still feel that testing should be performed please contact the laboratory to discuss testing of the sample.

NHS

UK Genetic Testing Network

UKGTN Testing criteria

Name of Disease(s):
LOEYS-DIETZ SYNDROME, TYPE 1A; LDS1A (609192)
LOEYS-DIETZ SYNDROME, TYPE 2A; LDS2A (608967)
LOEYS-DIETZ SYNDROME, TYPE 2B; LDS2B (610380)
LOEYS-DIETZ SYNDROME, TYPE 1B; LDS1B (610168)

Name of gene(s):
transforming growth factor, beta receptor 1; TGFBR1 (190181)
transforming growth factor, beta receptor II (70/80kDa); TGFBR2 (190182)

Patient name: **Date of birth:**

Patient postcode: **NHS number:**

Name of referrer:

Title/Position:
Lab ID:

Referrals will only be accepted from one of the following:

Referrer	Tick if this refers to you.
Consultant Clinical Geneticist	
Consultant Cardiologist	

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:

Criteria	Tick if this patient meets criteria
A patient should show at least two of the following features:	
• Dilatation of the aortic root/aortic dissection	
• Tortuosity or aneurysm of other arteries	
• Marfanoid body habitus	
• Craniofacial features such as craniosynostosis, hypertelorism, cleft palate/bifid uvula	
• Translucent skin	
Notes:	
1. Minimal diagnostic criteria for Loeys Dietz syndrome have not been established.	
2. All patients with dilatation of the aortic root/aortic dissection and Marfanoid body habitus should be evaluated for Marfan syndrome.	

If the sample does not fulfil the clinical criteria or you are not one of the specified types of referrer and you still feel that testing should be performed please contact the laboratory to discuss testing of the sample.

UKGTN Testing Criteria

Test name: **Loeys Dietz Syndrome**	
Approved name and symbol of disorder/condition(s): Loeys-Dietz Syndrome Type 3 and 4; LDS3 and LDS4	**OMIM number(s):** 613795, 614816
Approved name and symbol of gene(s): SMAD3, TGFB2	**OMIM number(s):** 603109, 190220

Patient name:	**Date of birth:**
Patient postcode:	**NHS number:**
Name of referrer:	
Title/Position:	**Lab ID:**

Referrals will only be accepted from one of the following:	
Referrer	**Tick if this refers to you.**
Consultant Clinical Geneticist	
Consultant Cardiologist in liaison with clinical geneticist	

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:	
Criteria	**Tick if this patient meets criteria**
Dilation of the aortic root / aortic dissection	
OR Tortuosity or aneurysm of other arteries	
OR At risk family members where familial mutation is known.	

If the sample does not fulfil the clinical criteria or you are not one of the specified types of referrer and you still feel that testing should be performed please contact the laboratory to discuss testing of the sample.

Approval Date: Sept 2013

NHS

UK Genetic Testing Network

UKGTN Testing criteria

Name of Disease(s): Marfan syndrome; MFS (154700)

Name of gene(s): fibrillin 1; FBN1 (134797)

Patient name: **Date of birth:**

Patient postcode: **NHS number:**

Name of referrer:

Title/Position:
Lab ID:

Referrals will only be accepted from one of the following:

Referrer	Tick if this refers to you.
Clinical Geneticist	
Consultant Cardiologist (Adult or Paediatric)	

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:

Criteria	Tick if this patient meets criteria
Suspected diagnosis of Marfan syndrome using revised Ghent criteria (Loeys 2010)*	
AND Dilated Aortic root **OR**	
Ectopia Lentis **OR**	
Family History of Suspected MFS **OR**	
Systemic score ≥ 7 (See Box for score)	
AND **Purpose for knowing mutation in this individual case must be one or more from list below;**	
- affects aortic screening /clinical management	
OR - allows prenatal testing	
OR - enables cascade family testing	
OR - avoids other investigation or seeking other clinical opinions for index case or relatives	
OR - enables targeting of clinical screening in relatives	
OR - provides knowledge of genetic risk	

If the sample does not fulfil the clinical criteria or you are not one of the specified types of referrer and you still feel that testing should be performed please contact the laboratory to discuss testing of the sample.

Approval Date: Sept 2012

REVISED GHENT CRITERIA (Loeys 2010)

*** Loeys BL et al. The revised Ghent nosology for the Marfan syndrome. Journal of Medical Genetics 2010; 47: 476-485. Doi:10.1136/jmg.2009.072785**

Table 1. Calculation of the Systemic Score

Feature	Value
Wrist AND thumb sign	3
Wrist OR thumb sign	1
Pectus carinatum deformity	2
Pectus excavatum or chest asymmetry	1
Hindfoot deformity	2
Plain flat foot (pes planus)	1
Pneumothorax	2
Dural ectasia	2
Protrusio acetabulae	2
Reduced upper segment / lower segment AND increased arm span/height ratios	1
Scoliosis or thoracolumbar kyphosis	1
Reduced elbow extension	1
3 of 5 facial features	1
Skin striae	1
Myopia	1
Mitral valve prolapse	1

Maximum total: 20 points
Score ≥ 7 indicates systemic involvement
US/LS= upper segment/lower segment ratio

Aortic root enlargement (Z-score ≥2.0 in those age ≥20 years or ≥3.0 in those age <20 years). Aortic size must be standardised to age and body size for accurate interpretation. A Z-score ≥2.0 infers a value at or above the 95th percentile, while a Z-score ≥3.0 infers a value at or above the 99th percentile.

UKGTN Testing Criteria

Approved name and symbol of disease/condition(s):	**OMIM number(s):**
Marfan Syndrome Type 1 (MFS)	154700
Approved name and symbol of gene(s):	**OMIM number(s):**
ACTA2, MYH11 TGFBR1, TGFRB2	160745, 102620, 190181, 190182

Patient name:	**Date of birth:**
Patient postcode:	**NHS number:**
Name of referrer:	
Title/Position:	**Lab ID:**

Referrals will only be accepted from one of the following:

Referrer	**Tick if this refers to you.**
Clinical Geneticist	
Consultant Cardiologist (Adult or Paediatric)	

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:

Criteria	**Tick if this patient meets criteria**
Suspected diagnosis of Marfan syndrome using revised Ghent criteria (Loeys 2010)*	
AND Dilated Aortic root **OR**	
Ectopia Lentis **OR**	
Family History of Suspected MFS **OR**	
Systemic score ≥ 7 (See Box for score)	
AND **Purpose for knowing mutation in this individual case must be one or more from list below;**	
- affects aortic screening /clinical management	
OR - allows prenatal testing	
OR - enables cascade family testing	
OR - avoids other investigation or seeking other clinical opinions for index case or relatives	
OR - enables targeting of clinical screening in relatives	
OR - provides knowledge of genetic risk	
AND FBN1 testing carried out and negative	
OR Family member with mutation in ACTA2/MYH11/TGFBR1/ TGFBR2	

Approval Date: Sept 2011 revised Nov 2013

If the sample does not fulfil the clinical criteria or you are not one of the specified types of referrer and you still feel that testing should be performed please contact the laboratory to discuss testing of the sample.

REVISED GHENT CRITERIA (Loeys 2010)

*** Loeys BL et al. The revised Ghent nosology for the Marfan syndrome. Journal of Medical Genetics 2010; 47: 476-485. Doi:10.1136/jmg.2009.072785**

Table 1. Calculation of the Systemic Score

Feature	Value
Wrist AND thumb sign	3
Wrist OR thumb sign	1
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Pectus excavatum or chest asymmetry	1
Hindfoot deformity	2
Plain flat foot (pes planus)	1
Pneumothorax	2
Dural ectasia	2
Protrusio acetabulae	2
Reduced upper segment / lower segment AND increased arm span/height ratios	1
Scoliosis or thoracolumbar kyphosis	1
Reduced elbow extension	1
3 of 5 facial features	1
Skin striae	1
Myopia	1
Mitral valve prolapse	1

Maximum total: 20 points
Score ≥ 7 indicates systemic involvement
US/LS= upper segment/lower segment ratio

Aortic root enlargement (Z-score ≥2.0 in those age ≥20 years or ≥3.0 in those age <20 years). Aortic size must be standardised to age and body size for accurate interpretation. A Z-score ≥2.0 infers a value at or above the 95th percentile, while a Z-score ≥3.0 infers a value at or above the 99th percentile.

Appendix 3

UKGTN testing criteria for inherited cardiomyopathies

1. Familial hypertrophic cardiomyopathy 16 gene panel
2. Familial dilated cardiomyopathy 28 gene panel
3. Arrhythmogenic right ventricular cardiomyopathy 8 gene panel

UKGTN Testing Criteria

Test name: Familial Hypertrophic Cardiomyopathy (HCM) 16 Gene Panel	
Approved name and symbol of disease/condition(s): See appendix 1 **Approved name and symbol of gene(s):** See appendix 1	**OMIM number(s):** **OMIM number(s):**

Patient name:	**Date of birth:**
Patient postcode:	**NHS number:**
Name of referrer:	
Title/Position:	**Lab ID:**

Referrals will only be accepted from one of the following:	
Referrer	**Tick if this refers to you.**
Consultant Clinical Geneticist	
Consultant Cardiologist (adult and paediatric) in liaison with Clinical Genetics Department	

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:	
Criteria	**Tick if this patient meets criteria**
Unexplained left ventricular hypertrophy (LVH) with a family history of LVH or Sudden Cardiac Death (SCD).	
Unexplained left ventricular hypertrophy (LVH) with no known family history	

Additional information:
At risk family members where familial mutation is known, do not require a full panel test but, should be offered analysis of the known mutation

HCM is a disease characterised by unexplained LV hypertrophy with non dilated ventricular chambers, in the absence of other cardiac or systemic disease that itself would be capable of producing the magnitude of hypertrophy evidence in a given patient.

If the sample does not fulfil the clinical criteria or you are not one of the specified types of referrer and you still feel that testing should be performed please contact the laboratory to discuss testing of the sample

Familial hypertrophic cardiomyopathy 16 gene panel

HGNC standard gene symbol	HGNC number	OMIM Number (gene)	OMIM standard name of condition	Mode of inheritance	OMIM number
ACTC1	143	*102540	Cardiomyopathy, familial hypertrophic,11	AD	612098
ACTN2	164	*102573	-	AD	-
ANKRD1	15819	*609599	-	AD	-
CSRP3	2472	*600824	Cardiomyopathy, familial hypertrophic, 12	AD	612124
FHL1	3702	*300163	-	X-Linked	-
GLA	4296	*300644	Fabry Disease, cardiac variant	X-Linked	301500
LAMP2	6501	*309060	-	X-Linked	300257
MYBPC3	7551	*600958	Cardiomyopathy, familial hypertrophic, 4	AD	115197
MYH7	7577	*160760	Cardiomyopathy, familial hypertrophic, 1	AD	192600
MYL2	7583	*160781	Cardiomyopathy, familial hypertrophic, 10	AD	608758
MYL3	7584	*160790	Cardiomyopathy, familial hypertrophic, 8	AD	608751
PLN	9080	*172405	Cardiomyopathy, familial hypertrophic, 18	AD	613874
PRKAG2	9386	*602743	Cardiomyopathy, familial hypertrophic, 6	AD	600858
TNNI3	11947	*191044	Cardiomyopathy, familial hypertrophic, 7	AD	613690
TNNT2	11949	*191045	Cardiomyopathy, familial hypertrophic, 2	AD	115195
TPM1	12010	*191010	Cardiomyopathy, familial hypertrophic, 3	AD	115196

NHS

UK Genetic Testing Network

UKGTN Testing Criteria

Test name: **Familial Dilated Cardiomyopathy (DCM) 28 Gene Panel**	
Approved name and symbol of disorder/condition(s): See appendix 1	**OMIM number(s):**
Approved name and symbol of gene(s): See appendix 1	**OMIM number(s):**

Patient name:	**Date of birth:**
Patient postcode:	**NHS number:**
Name of referrer:	
Title/Position:	**Lab ID:**

Referrals will only be accepted from one of the following:	
Referrer	**Tick if this refers to you.**
Consultant Clinical Geneticist	
Consultant Cardiologist	

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:	
Criteria	**Tick if this patient meets criteria**
Imaging evidence of left ventricular dilatation and systolic dysfunction (ejection fraction less than 50% and non-genetic causes excluded)	

Additional Information:

For panel tests:
At risk family members where familial mutation is known do not require a full panel test but should be offered analysis of the known mutation

If the sample does not fulfil the clinical criteria or you are not one of the specified types of referrer and you still feel that testing should be performed please contact the laboratory to discuss testing of the sample.

Approval Date: Sept 2013

Familial dilated cardiomyopathy 28 gene panel

HGNC standard gene symbol	HGNC number	OMIM Number (gene)	OMIM standard name of condition	Mode of inheritance	OMIM number
ACTC1	143	*102540	Cardiomyopathy, dilated, 1R	AD	613424
ACTN2	164	*102573	Cardiomyopathy, dilated, 1AA	AD	612158
ANKRD1	15819	*609599	-	AD	-
CRYAB	2389	*123590	Cardiomyopathy, dilated, 1II	AD	615184
CSRP3	2472	*600824	Cardiomyopathy, dilated, 1M	AD	607482
DES	2770	*125660	Cardiomyopathy, dilated, 1I	AD	604765
DSC2	3036	*125645	-	AD	610476
DSG2	3049	*125671	Cardiomyopathy, dilated, 1BB	AD	612877
DSP	3052	*125647	Dilated cardiomyopathy with woolly hair and keratoderma	AD, AR	605676
FHL1	3702	*300163	-	X-Linked	300696
FHL2	3703	*602663	-	AD	-
GLA	4296	*300644	Fabry disease, cardiac variant	X-Linked	301500
JUP	6207	*173325	-	AD	-
LAMP2	6501	*309060	-	X-Linked	300257
LMNA	6636	*150330	Cardiomyopathy, dilated, 1A	AD	115200
MYBPC3	7551	*600958	Cardiomyopathy, dilated, 1MM	AD	615396
MYH7	7577	*160760	Cardiomyopathy, dilated, 1S	AD	613426
MYL2	7583	*160781	-	AD	-
MYL3	7584	*160790	-	AD	-
PKP2	9024	*602861	-	AD	-
PLN	9080	*172405	Cardiomyopathy, dilated, 1P	AD	609909
PRKAG2	9386	*602743	-	AD	-
SCN5A	10593	*600163	Cardiomyopathy, dilated, 1E	AD	601154
TMEM43	28472	*612048	-	AD	-
TNNI3	11947	*191044	Cardiomyopathy, dilated, 1FF Cardiomyopathy, dilated, 2A	AD	613286 611880
TNNT2	11949	*191045	Cardiomyopathy, dilated, 1D	AD	601494
TPM1	12010	*191010	Cardiomyopathy, dilated, 1Y	AD	611878
TTN	12403	*188840	Cardiomyopathy, dilated, 1G	AD	604145

NHS

UK Genetic Testing Network

UKGTN Testing Criteria

Test name: **Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC) 8 Gene Panel**	
Approved name and symbol of disorder/condition(s): See appendix 1	**OMIM number(s):**
Approved name and symbol of gene(s): See appendix 1	**OMIM number(s):**

Patient name:	**Date of birth:**
Patient postcode:	**NHS number:**
Name of referrer:	
Title/Position:	**Lab ID:**

Referrals will only be accepted from one of the following:

Referrer	**Tick if this refers to you.**
Consultant Clinical Geneticist	
Consultant Cardiologist	

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:

Criteria	**Tick if this patient meets criteria**
TWO of : **1.** RV dilatation, functional impairment, or localised RV aneurysm, in the absence of similar LV dysfunction. **2.** Fibrofatty replacement of myocardium seen on biopsy **3.** ECG shows prolongation of QRS focally in leads V1-V3 **4.** Family history of definite ARVC detected at autopsy/surgery	
OR ONE of above, AND ONE OR MORE OF : **4.** Mild RV dilatation, impairment, or focal RV hypokinesis in presence of normal LV. **5.** ECG shows inverted T waves in V2, V3, in absence of RBBB, OR shows signal-averaged late potential. **6.** LBBB-type VT, OR frequent Vent.ectopics (>1000/24hrs) **7.** Close F.Hist. of sudden cardiac death <35yrs, suspected as ARVD	
OR NONE OF 1-3, but ALL of 4-7.	

Additional Information:

For panel tests:

At risk family members where familial mutation is known do not require a full panel test but should be offered analysis of the known mutation

If the sample does not fulfil the clinical criteria or you are not one of the specified types of referrer and you still feel that testing should be performed please contact the laboratory to discuss testing of the sample.

Approval Date: Sept 2013

Arrhythmogenic right ventricular cardiomyopathy 8 gene panel

HGNC standard gene symbol	HGNC number	OMIM Number (Gene)	OMIM standard name of condition	Mode of inheritance	OMIM number
DES	2770	*125660		AD	-
DSC2	3036	*125645		AD	-
DSG2	3049	*125671	Arrhythmogenic right ventricular dysplasia, 10	AD	612877
DSP	3052	*125647	Arrhythmogenic right ventricular dysplasia, 8	AD, AR	607450
JUP	6207	*173325	Arrhythmogenic right ventricular dysplasia, 12 Naxos disease	AD, AR	611520 601214
LMNA	6636	*150330		AD	-
PKP2	9024	*602861	Arrhythmogenic right ventricular dysplasia, 9	AD	609040
TMEM43	28472	*612048	Arrhythmogenic right ventricular dysplasia, 8	AD	604400

Appendix 4

UKGTN testing criteria for familial hypercholesterolaemia

1. Familial hypercholesterolaemia 4 gene panel

UKGTN Testing Criteria

Test name: **Familial Hypercholesterolaemia 4 gene Panel**	
Approved name and symbol of disorder/condition(s): See appendix 1	**OMIM number(s):**
Approved name and symbol of gene(s): See appendix 1	**OMIM number(s):**

Patient name:	**Date of birth:**
Patient postcode:	**NHS number:**
Name of referrer:	
Title/Position:	**Lab ID:**

Referrals will only be accepted from one of the following:	
Referrer	**Tick if this refers to you.**
Clinical Geneticists	
Consultant Lipidologist	
Consultant in Metabolic Medicine	
Consultant Cardiologist	

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:	
Criteria	**Tick if this patient meets criteria**
Simon Broome Criteria for **definite FH** in adults*	
Simon Broome Criteria for **possible FH** in adults*	
Total or LDL-C above the 95th percentile for age and gender in children	
Family history of confirmed familial hypercholesterolaemia (provide details of mutation, family relationship and testing laboratory)	

*For mutation screen Simon Broome diagnostic criteria for probands

Definite familial hypercholesterolaemia is defined as:

1. Total cholesterol above 6.7mmol/l or LDL cholesterol above 4.0mmol/l in a child aged under 16 years or total cholesterol above 7.5mmol/l or LDL cholesterol above 4.9mmol/l in an adult (levels either pre-treatment or highest on treatment) **and**

2. Tendon xanthomas in patient, or in 1st degree relative (parent, sibling, child), or in 2nd degree relative (grandparent, uncle, aunt) **OR**

Approval Date: Mar 2015

3. DNA-based evidence of an LDL receptor mutation, familial defective apo B-100, or a PCSK9 mutation.

Possible familial hypercholesterolaemia is defined as no.1 above and to include one of the criteria below:

1. Family history of myocardial infarction: below age of 50 years in 2nd degree relative or below age 60 years in 1st degree relative

2. Family history of raised total cholesterol: above 7.5mmol/l in adult 1st or 2nd degree relative or above 6.7mmol/l in child or sibling aged under 16 years.

Additional Information:
For panel tests:
At risk family members where familial mutation is known do not require a full panel test but should be offered analysis of the known mutation

If the sample does not fulfil the clinical criteria or you are not one of the specified types of referrer and you still feel that testing should be performed please contact the laboratory to discuss testing of the sample.

Appendix 1

HGNC standard name and symbol of the gene	HGNC number	OMIM number	OMIM standard name of condition	OMIM number	% of horizontal coverage of gene	MLPA
Low density lipoprotein receptor - LDLR	6547	606945	Hypercholesterolemia, familial	143890	100%	N/A
Apolipoprotein B - APOB	603	107730	Hypercholesterolemia, familial Hypercholesterolemia, autosomal dominant, type B	143890 144010	100%	N/A
Proprotein Convertase subtilisin/kexin type 9 - PCSK9	20001	607786	Hypercholesterolemia, familial Hypercholesterolemia, autosomal dominant, 3, HCHOLA3	143890 603776	100%	N/A
Low density lipoprotein receptor adaptor protein 1- LDLRAP1	18640	605747	Hypercholesterolemia, autosomal recessive (ARH)	603813	100%	N/A

Approval Date: Mar 2015

Appendix 5

British Cardiovascular Society Annual Conference

Manchester Central, 8th June 2015

UKGTN/BHF sessions programme

The new cardiac genetic testing panels: implications for the clinical cardiologist

Session 1

Chairs: Professor Jeremy Pearson, Professor Nilesh Samani

1. The emergence of new genetic tests for cardiac disease; what the cardiologist needs to know
   Professor Clifford Garratt
2. The UKGTN and the evaluation process
   Dr Shehla Mohammed
3. Sudden cardiac death syndrome 62 gene panel
   Dr Kay Metcalfe
4. Familial thoracic aortic aneurysm syndromes and Marfan syndrome
   Dr Paul Clift

Session 2

Chairs: Dr Fiona Stewart, Professor Perry Elliott

1. Inherited cardiomyopathies 28 gene panel
   Professor Hugh Watkins
2. Familial hypercholesterolaemia gene pane testing: closing the gap in ascertainment
   Dr Maggie Williams
3. The role of whole genome sequencing in cardiovascular disorders
   Professor Bernard Keavney

UK Genetic Testing Network

www.ukgtn.nhs.uk

UK Genetic Testing Network (UKGTN)
c/o South East Commissioning Support Unit (SE CSU)
15 Marylebone Rd
London
NW1 5JD

Tel 020 3350 4999
Fax 020 3350 4458