The Cardiac Society of Australia and New Zealand

Guidelines for Genetic Testing of Inherited Cardiac Disorders

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Introduction

In recent years, there has been increasing recognition of the importance of inherited gene mutations as a cause of cardiomyopathies and arrhythmogenic diseases. Substantial progress has been made in elucidating the molecular defects underpinning these diseases, but the impact of these discoveries on clinical management is often unclear. This document aims to outline the current status of genetic testing for a range of inherited cardiac disorders in order to provide cardiologists and other health professionals with a clear understanding of the process, including the advantages and limitations of testing.

Indications for Genetic Testing

There are a number of inherited cardiac disorders for which genetic causes have been identified, including hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), arrhythmogenic right ventricular cardiomyopathy (ARVC), left ventricular non-compaction, restrictive cardiomyopathy, long QT syndrome (LQTS), Brugada syndrome (BrS), catecholaminergic polymorphic ventricular tachycardia (CPVT) and atrial fibrillation. Genetic testing is also indicated in some multisystem disorders with frequent cardiovascular involvement, such as familial hypercholesterolaemia (FH) and Marfan syndrome (MS). Genetic testing for HCM, LQTS, and FH has become an integral part of clinical management[1,2], however genetic testing is not so straightforward for many of the other conditions.

Genetic testing is the process of searching through the DNA sequence of disease-associated genes to identify a sequence variation that is pathogenic ("mutation"). In general, genetic testing is considered when an individual has a clinical diagnosis of an inherited heart disease, particularly if there is a positive family history. Initial genetic testing to identify a causative mutation is ideally offered to a member of the family who has definite clinical disease to maximise the chance of detecting a mutation. In cases where the only known affected individual is deceased there may be the option of testing post-mortem blood/tissue samples or Guthrie card, however the quality of DNA being tested is often diminished making this technically challenging. It is now well established that a small proportion of families have more than one disease-causing mutation, in either different alleles of the same gene, or in different genes. This phenomenon is most widely reported in HCM, LQTS and ARVC[3-9]. Therefore, it is recommended that for these conditions, the search for the family-specific mutation in the index case should not cease once a mutation is identified, but should continue to include all of the common genes associated with the condition. This also highlights the advantages of testing large gene panels rather than single genes.

Availability of Genetic Testing

Genetic testing for inherited cardiac disorders is available through clinical genetics services throughout Australia. A small number of specialised cardiac genetic clinics operate in major centres including Sydney, Melbourne and Brisbane and these comprise a multidisciplinary team of cardiologists, clinical geneticists and genetic counsellors/coordinators[10]. Some DNA testing is performed within Australian laboratories, however testing for many conditions requires sending samples to overseas laboratories (see Appendix).

Several factors can impact on the availability and utility of genetic testing, such as access to testing, cost of testing and the mutation detection rate. These factors vary greatly depending on the disease (see Table 1), with genetic testing for HCM, LQTS, and FH being readily available and having a relatively high probability (up to 75%) of finding a mutation. For other conditions such as DCM, there is a low yield of mutations found overall. However, mutation screening of the *LMNA* gene is worthwhile in the subset of families with DCM and conduction-system disease[11,12]. For other families with DCM, participation in research studies investigating new candidate genes could be considered as an option.

Families with a diagnosis of an inherited heart disease should be referred to either their local clinical genetics service, or one of the major cardiac genetic clinics. Health professionals who coordinate and manage these clinics are up-to-date with the laboratories offering the most competitive service and price, and are able to offer appropriate genetic counselling. Genetic testing performed by commercial laboratories does not yet attract any Medicare rebate. Reimbursement of costs may be covered in some areas by clinical genetics or cardiology services, or charged to family members and needs to be explored on an individual basis. Cost recovery for genetic testing is a subject of ongoing discussion and rebates for this service are likely to be available in the future.

Interpretation of genetic test results

There are several possible outcomes of the initial genetic testing performed in the affected member of a family. Firstly, a potential disease-causing mutation may be identified. With recent technological advances that enable high-throughput sequencing of multiple genes, a large number of sequence variants may be found in this individual's DNA sample. The majority of these are likely to be missense (that is, a single base pair substitution resulting in a change in amino acid), and there may be a number of novel variants. It is common practice for laboratories to report all variants found in genetic testing, including novel and previously reported variants. A number of criteria are used to support a variant as being potentially disease-causing, including segregation with disease status in other affected family members, absence from a control population, and location in a conserved amino acid sequence[13]. Evidence of altered gene function is ideally required to confirm pathogenicity, however this type of supporting evidence is frequently not available. Other types of mutations, for example, those that lead to shortened (truncated) gene products or involving deletions and duplications of large segments of a gene, are generally easier to classify as pathogenic. A number of new techniques are now available for detection of these copy number variations. Careful evaluation of data obtained from genetic testing is required to avoid giving misleading and incorrect genetic results. Interpretation of DNA sequence results should be undertaken by experienced personnel, including molecular geneticists, molecular cardiologists, clinical geneticists, and/or genetic counsellors.

Another common outcome of genetic testing is that a sequence variation of unknown significance may be identified. These are variations in the DNA for which there is not enough evidence to clearly categorize the variant as disease-causing. When a variation of unknown significance is identified, its utility in confirming the diagnosis in a proband with suspected disease is limited and it cannot be used for predictive gene testing in at-risk relatives.

Finally, in a large number of families, no mutation may be identified. There could be several explanations for this. It is possible that the disease-causing mutation in the family is present in a gene that was not tested, or in a gene that is yet to be discovered. Alternatively, it is possible that there is a mutation in one of the genes that was tested, but the mutation detection technique employed failed to detect the mutation. For example, gene sequencing is considered the "gold standard" for mutation detection, but even gene sequencing does not detect large deletions or duplications within genes. Laboratory reports should state the testing technique(s) used and the estimated sensitivity and specificity of each.

Impact of identifying a mutation for families and predictive testing

Identification of a mutation in a family often provides an explanation for why the disease has occurred. It also allows for cascade testing of other affected and unaffected family members.

Testing of affected family members is performed as a confirmation of their disease status and to exclude the possibility of a "phenocopy" (that is, an individual who has an acquired rather than genetic cause of the same condition as other members of a family). This enables an accurate risk assessment to be given for their offspring.

Asymptomatic family members can be offered a predictive genetic test to clarify whether they are at risk of developing clinical disease and to determine the inheritance risk to their children. There may also be implications for participation in sports and employment. The decision to have predictive genetic testing is complex and should be made in the context of a clinical genetics service offering pre- and post-test genetic counselling. Pre-test counselling focuses on informing the patient about the testing process and should include a discussion of the potential effects of a positive or negative test result. The genetic counselling process includes obtaining the patient's informed consent, and should facilitate autonomous decision-making. Discussion of a possible positive (presence of mutation) or negative (absence of mutation) genetic result should examine the impact on family dynamics, personal coping mechanisms, as well as the potential psychological or emotional impact of testing[14]. The impact of a positive genetic test result on the ability to acquire insurance, particularly life insurance, is an important point to raise.

A positive result may remove uncertainty and have a favourable psychosocial impact. However, for some individuals, a positive result may be particularly distressing, evoking feelings of anxiety, anger, fear, and guilt, particularly for parents of children undergoing gene testing[15]. More research on the psychological impact of predictive gene testing for cardiovascular genetic conditions is needed to guide genetic counselling. The few studies that have been performed have shown that psychological outcomes (for example, anxiety and depression, measured by standardised scales) are similar for those that test positive when compared to their mutation-negative counterparts[16] or to population controls[17]. These results are largely in keeping with reports on the impact of predictive testing in adult onset neurological diseases and familial cancers[18,19]. Interestingly, partners of individuals whose predictive gene test was positive had higher levels of anxiety compared to partners of non-carriers[16], reinforcing the importance of including partners in the predictive testing counselling, not only to support the person being tested, but also to have their own concerns addressed. A major concern for those individuals who receive a positive result may be the fear of having passed on the mutation to offspring. It could be helpful to emphasise the benefits in this situation, that is, that precise genetic risk information can be provided to their descendents and for those that test positive, clinicians are able to guide the frequency of clinical follow-up. A positive predictive result also enables detection of early disease in some cases, along with an opportunity for prophylactic treatment and lifestyle modification, as appropriate[20]. For most inherited cardiac diseases, however, given there may be significant intra-familial phenotypic variation, there is still a lot of uncertainty regarding age of onset, progression of disease and benefits of prophylactic treatment.

A negative result, on the other hand, can provide reassurance and eliminates the need for cardiac follow-up. In the event of a new onset of symptoms, the possibility of an additional cause of disease in the family should be considered. Receiving a negative test result may also generate an emotional reaction, such as guilt, particularly in families where other siblings are affected or mutation-positive. Ongoing counselling follow-up, either with a genetic counsellor or through another qualified counsellor, should be offered where required.

Genetic testing in children

Predictive genetic testing in children is likewise a complex issue, and is managed on a case-by-case basis, and varies with the condition for which predictive testing is being requested.

The Human Genetics Society of Australasia guidelines on predictive genetic in children and adolescents state that when there is medical benefit in the immediate future genetic testing should be offered[21]. While cardiovascular genetic diseases may fit these criteria particularly with regard to avoiding high-level sports for the risk of sudden death, consideration must be made in view of the child and their current and future autonomy. For the majority of patients at risk of a familial cardiomyopathy, consideration should be given to delaying testing to give the child an opportunity to have some understanding of the test and the implications of its result. This is generally from the early to mid-teens as this coincides with the period of onset of disease when regular clinical screening begins for most cardiomyopathies. For inherited arrhythmia syndromes, predictive testing may be performed earlier in life given that sudden death can occur at any age and for most of these syndromes, for example, LQTS and CPVT, prophylactic therapy may be initiated. Genetic testing of minors may include assessment by a child mental health specialist to ensure that a genetic result will not adversely affect the well being of the child.

Prenatal testing

If a disease-causing mutation is known in the family, this information can be used for future/current pregnancies. In established pregnancies, genetic testing of foetal DNA via chorionic villus sampling (10-12 weeks gestation) or amniocentesis (15-18 weeks gestation) can determine if the foetus carries the disease-causing gene, and therefore may develop disease in the future. If parents receive a positive result they can choose whether or not to continue with the pregnancy. A couple wishing to consider pre-natal diagnosis should be referred to their local genetics unit with experience in genetic testing in pregnancy. Pre-natal diagnosis is not available in families where the causative mutation is not known.

Pre-implantation genetic diagnosis (PGD) is a method using in vitro fertilisation (IVF) technologies, where embryos are tested for the presence of the disease-causing mutation. Only those embryos without the mutation are implanted. PGD is an expensive technology, as each family's mutation needs to be worked up by the service before beginning IVF. Families who are interested in pursuing PGD should be referred to an appropriate local IVF provider, who will discuss with them the process, cost and success rates.

Implications for patient management

In most cases, the presence of a mutation on genetic testing result will not alter the clinical management of an individual who is already known to be affected. However, in cases where an unaffected individual receives a positive predictive gene test result, an ongoing surveillance programme should be recommended. Due to variable age of onset, surveillance should be started early, and may be determined by the natural history in the family. Genotype-phenotype correlations, if known, could be an important consideration in determining screening for asymptomatic mutation positive individuals. In some conditions, such as HCM, ARVC or CPVT, a positive family history of sudden death, may lower the threshold for preventive implantation of an automated implantable cardioverter defibrillator (AICD)[20].

Pregnancy management of women with a positive genetic test result is an important consideration. For affected women, who are undergoing pharmacological treatment, and contemplating pregnancy, a specialist review is recommended. An evaluation of the benefits and harms of cessation of medication is needed, or alternatively, consideration of drugs that will minimise the chances of an unsuccessful pregnancy. During pregnancy, there is a need for regular cardiac evaluation, as pregnancy may worsen the condition for an already affected individual, or may even unmask disease in an as yet unaffected individual, and result in early presentation[22].

Implications for lifestyle recommendations

Mutation-positive individuals should receive appropriate lifestyle modification advice as part of result follow-up. Strenuous competitive sporting activities should be avoided in individuals with inherited cardiac disorders[23]. Information should be provided to highlight the deleterious effects of alcohol excess, as well as arrhythmic triggers such as recreational drugs. Cessation of smoking is generally recommended, and is particularly important in families with FH.

Conclusion

Genetic testing for inherited cardiac disorders has seen major advancements in recent years. Several factors such as access, cost, utility and yield of testing should be considered when offering genetic testing for a particular condition.

In summary, we make the following "who-to-test" recommendations:

- Genetic testing should be discussed with all clinically-affected probands who have the inherited heart diseases outlined in this document. Probands and referring physicians should have realistic expectations about the likely yield of testing, which currently ranges from high, as is the case for HCM, LQTS, FH, and CPVT, to relatively low (e.g. DCM). Genetic testing should be performed in the setting of a specialised cardiac genetic clinic or clinical genetics service where appropriate genetic counselling can be offered.

- Asymptomatic first-degree family members should attend a specialised cardiac genetic clinic or their local cardiologist for periodic clinical screening as per current guidelines. If a likely-pathogenic gene variation is identified in a family proband, symptomatic and asymptomatic family members should be informed about the possibility of genetic testing and appropriate genetic counselling provided.

Although genetic testing plays an important role in family management, genetic test results should not be used as a surrogate for careful clinical evaluation of family members, which remains the primary basis for a diagnosis of inherited heart disease. Detailed phenotype evaluation and genotype-phenotype correlations may help to determine which patients are most likely to benefit and which genes are likely to have the highest yield for testing. Clinical genetic services and specialist cardiac genetic clinics around Australia are best-equipped to see patients and discuss these issues as well as important psychosocial considerations prior to commencing genetic testing. For those conditions for which relatively less is known about the genetic causes of disease, participation in genetics research programs is a worthwhile option and should be encouraged. The introduction of new technologies for mutation detection, such as genome-wide sequencing promises to rapidly increase the rate of variant detection and should enable more families to acquire genotype results. However, this will bring new challenges for sequence variant interpretation and many variants of uncertain significance are likely to be found. As this is a rapidly expanding field, recommendations for genetic testing will change considerably over the next few years and we encourage readers to consult with their local cardiac genetic service for the most up-to-date information.

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Table 1. Overview of currently available genetic testing in inherited heart disease

Disease	Genes tested	Detection rate[a]	Approximate cost[a] (AUD)	Ref.
HCM	*MyH7, MyBPC, TNNT2, TNNI3, TPM1, MYL2, MYL3, ACTC1, CSRP3*[b]	40-60%	$2000-$3500	11
DCM	Various combinations of*:* *ABCC9, ACTC, ACTN2, ANKRD1, CSRP3, CTF1, DES, DSG2, DSP, EMD, LDB3, LMNA, MYBPC3, MYH7, PLN, SCN5A, SGCD, TAZ, TCAP, TNNC1, TNNT2, TNNI3, TPM1, VCL.*	<30%	Up to $6,000	11,12
ARVC	*DSP, DSG2, DSC2, PKP2*	30-40%	$2000-$3000	11
LQTS	*KCNQ1* (LQT1), *KCNH2* (LQT2), *SCN5A* (LQT3), *KCNE1* (LQT5), *KCNE2* (LQT6), *KCNJ2* (LQT7)	75-80%	$2000-$3500	24
BrS	*SCN5A* (LQT3)	20-25%	$1800	25
CPVT	*RYR2, CASQ2*[c]	50-60%	$460- $1500	26,27
FH	*LDL-R, Apo-B, PCSK9*	25-75%	$1,000-$3,000	28
MS	*FBN1, TGFBR1, TGFBR2*	80-85%	$2,500	29,30

[a] Costs and detection rate vary by laboratory and represent initial testing of the index case in each family; costs of testing other family members will be less; see individual laboratory information for cost updates. [b] Some HCM gene testing panels will involve the *LAMP2*, *PRKAG2* and *GLA* genes (mutations in these genes can mimic HCM); [c] *CASQ* mutations causing CPVT are autosomal recessive. ARVC, arrhythmogenic right ventricular cardiomyopathy; BrS, Brugada Syndrome; CPVT, catecholaminergic polymorphic ventricular tachycardia; DCM, dilated cardiomyopathy; FH, familial hypercholesterolaemia; HCM, hypertrophic cardiomyopathy; LQTS, long QT syndrome; MS, Marfan syndrome.

APPENDIX: Useful contacts

FH Patient Support Group:
www.familialhypercholesterolaemiasupportwa.websyte.com.au
www.geneticsupportcouncil.org.au

A list of clinical genetics services throughout Australia and New Zealand can be found at:
www.genetics.com.au