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Use of Contemporary Genetics in Cardiovascular Diagnosis

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Abstract

An explosion of knowledge regarding the genetic and genomic basis for rare and common diseases has provided a framework for revolutionizing the practice of medicine. Achieving the reality of a genomic medicine era requires that basic discoveries are effectively translated into clinical practice through implementation of genetic and genomic testing. Clinical genetic tests have become routine for many inherited disorders and can be regarded as the standard-of-care in many circumstances including disorders affecting the cardiovascular system. New, high-throughput methods for determining the DNA sequence of all coding exons or complete genomes are being adopted for clinical use to expand the speed and breadth of genetic testing. Along with these extraordinary advances have emerged new challenges to practicing physicians for understanding when and how to use genetic testing along with how to appropriately interpret test results. This review will acquaint readers with general principles of genetic testing including newer technologies, test interpretation and pitfalls. The focus will be on testing genes responsible for monogenic disorders and on other emerging applications such as pharmacogenomic profiling. The discussion will be extended to the new paradigm of direct-to-consumer genetic testing and the value of assessing genomic risk for common diseases.

Keywords

genetic testing; genes; mutation; polymorphism; pharmacogenomics

Logistical Considerations for Genetic Testing

Genetic testing is a specialized diagnostic procedure that can be performed by commercial and research laboratories. However, in the United States, clinical genetic testing laboratories must meet stringent criteria for quality standards that conform to the federal Clinical Laboratory Improvement Amendments (CLIA).[1] Most research laboratories operate without CLIA certification, and data generated in this setting are not strictly appropriate for inclusion in patient medical records or for making clinical decisions. Discoveries made by research laboratories should be confirmed by a CLIA-certified clinical genetics laboratory if the results are meaningful to patient care.

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Unlike commonly used laboratory assays, genetic testing should be performed after informing the patient about the potential risks, benefits and limitations. Involvement of a genetic counselor is ideal in circumstances where either physician time or knowledge is limited. The potential negative impact of learning the results of a genetic test must be anticipated. Patients should be properly educated and carefully counseled about their long-term risks of having a genetic condition without inciting excessive apprehension by implying that genotype is an absolute predictor of disease. Physicians should also be sensitive to the potential socioeconomic fallout (e.g., insurability) from a genetic diagnosis and vigorously guard confidentiality of test results. In the United States, the Genetic Information Non-discrimination Act (GINA) prohibits workplace and health insurance discrimination based on genetic predisposition, but this sensitive information should be protected nonetheless.

Indications for Genetic Testing

Genetic testing is performed under a variety of circumstances and applied at various time points from before life begins to after death occurs (**Table 1**). The most common scenarios for clinical genetic testing for cardiovascular disorders occur beyond the perinatal period and include diagnostic, presymptomatic and postmortem testing. Diagnostic testing undertakes the primary discovery of genetic defects by screening a panel of genes previously associated with a specific disease. Directed genetic testing (e.g., detection of specific mutation or genomic variant) is performed when there is a known familial risk for a disease and a previously identified mutation in the parents or other first degree relative. Postmortem genetic testing is increasingly performed in the evaluation of sudden unexplained death especially in the young and yields positive findings in up to a quarter of autopsies.[2,3] Preimplantation genetic testing is performed on *in vitro* fertilized embyros to guide selection of unaffected embryos for transfer to a recipient uterus.[4] Although risk for cardiovascular disorders is not typically determined, there have been successful uses of preimplantation genetic diagnosis in cases of Holt-Oram syndrome[5,6] and familial cardiomyopathy.[7] Direct-to-consumer genetic testing is discussed later in the article.

A freely available internet resource, GeneTests™, provides a searchable database of clinical genetic testing laboratories, specialty clinics and other relevant information (**Table 2**).[8] The current database consists of entries from more than 600 laboratories worldwide offering tests for an aggregate of more than 3,000 genetic conditions. A variety of test types are indexed including molecular (e.g., DNA sequence), cytogenetic and biochemical assays. The clinic directory has information for more than 1,000 genetics clinics internationally. GeneTests™ can be searched using disease or gene names to retrieve a listing of laboratories performing specific tests along with their contact information. The database can also be searched to find laboratories and specialty clinics within a specific region.

Information in GeneTests™ has recently transitioned to a resource hosted by the National Center for Biotechnology Information (NCBI), the Genetic Testing Registry, which is a repository for testing information provided by laboratories. Both GeneTests™ and the Genetic Testing Registry provide gene and disease specific information derived in part from another resource, GeneReviews®, a collection of expertly authored and peer-reviewed

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articles on genes and genetic disorders. This site is also hosted by NCBI. A similar searchable database, the United Kingdom Gene Testing Network, provides online access to a catalog of genetic tests provided by clinically accredited laboratories in the UK.

There are several monogenic diseases primarily or secondarily affecting the cardiovascular system (**Table 3**). For many cardiovascular disorders in which Mendelian inheritance has already been established, genetic testing may already be indicated, and there have been published guidelines for the use and interpretation of genetic tests for many specific disorders including channelopathies and familial cardiomyopathies.[9,10] In other circumstances, an important step in determining which specific patients will benefit most from a genetic work-up is distinguishing cases that arise from a single gene mutation or definable genomic defect from those with a more complex etiology (e.g., combined impact of multiple genetic, developmental and environmental factors). Clinical evidence suggesting a single genetic locus etiology may include: 1) familial segregation of the trait in a pattern consistent with Mendelian inheritance (e.g., autosomal dominant, autosomal recessive or X-linked), 2) extreme phenotype with either unusual severity or early age of onset, and 3) associated clinical features that suggest a specific syndrome.

Recognizing Mendelian inheritance patterns requires the ascertainment of a thorough and reliable family history with construction of a multi-generation pedigree. However, this task requires considerable time and may not be practical for busy clinicians. Therefore, alternative strategies for acquiring family history data that involve properly trained allied health professionals (e.g., genetic counselors, nurse practitioners, physician assistants) who are familiar with the phenotype, computer or internet resources developed for collecting family health history, or carefully compiled disease-specific survey tools should be used when physician time is limited. Nuances of Mendelian inheritance such as incomplete penetrance (e.g., presence of a mutation does not correlate with disease in all cases) and subclinical disease expression may confound interpretation of pedigree data. For these reasons, involvement of genetic counselors or referral to a medical geneticist is highly recommended.

Extreme phenotypes may suggest a monogenic disorder even in the absence of a clear family history, such as with *de novo* mutations. Unusually early onset of phenotypes that are more typically adult-onset such as hypertensive stroke, coronary artery disease, heart failure and sudden cardiac death should raise suspicion of a monogenic condition. Additional clinical features, which may affect organs or tissues outside the cardiovascular system, may herald the presence of a specific genetic or genomic syndrome.

Genetic testing may have value beyond establishing or confirming a particular diagnosis in just the primary patient. Demonstrating a specific mutation or genomic defect provides an opportunity to offer targeted testing to relatives who may be affected by the same disease or are at-risk based on their shared genetic makeup. Collateral testing of first degree relatives (e.g., siblings, parents, offspring), referred to as ‘cascade’ screening, can identify pre-symptomatic individuals who may benefit from additional diagnostic procedures or prophylactic therapy. Positive genetic test results may also help tailor therapy and provide a basis for reproductive genetic counseling.

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Next-generation Clinical Genetic Testing

An extensive technological repertoire exists to test DNA for a range of medically relevant genetic and genomic defects ranging from single nucleotide variants to chromosomal aberrations. Recent advances in DNA sequencing technology have greatly expanded the scope and capabilities of clinical genetic testing laboratories to discovery medically actionable mutations in cardiovascular disorders.[11,12] Until the past few years, the standard sequencing platforms used the Sanger method and this approach served as the 'workhorse' for sequencing the human genome. The advent of next-generation sequencing brings a paradigm-shift in the scale and complexity of clinical genetic testing enabling laboratories to perform testing on large panels of disease-relevant genes, all coding exons (exome) or whole genomes. This has allowed genetic diagnoses in small families with rare disorders and discovery of novel disease-causing genes at much lower cost per nucleotide. In 2013, the Food and Drug Administration (FDA) granted marketing authorization for the first next-generation sequencer to be used for clinical genetic testing.[13] During the past 3 years, there has been an explosion in the number of reports demonstrating successful uses of exome and genome sequencing to uncover the genetic basis for several disorders with cardiovascular manifestations in the research setting (**Supplemental Table S1**). Accompanying these advances are new challenges for handling and interpreting massive quantities of data as well as managing discovery of medically actionable incidental findings.

Sequence capture technology coupled with next-generation sequencing has enabled two new genetic testing paradigms: simultaneous sequencing of multiple genes, and whole-exome sequencing.[14] **Figure 1** illustrates the general workflow of these approaches. Initially, genomic DNA is sheared into small fragments (typically 200-500 bp), then these fragments of DNA are mixed with a capture reagent. The capture reagent is a solution mixture of thousands of synthetic DNA or RNA molecules designed with base pair complementarity to all sequences within the target of interest (e.g., all exons of a candidate gene panel, or all coding exons in the genome).[12] For exome sequencing, the capture reagent will generally target approximately 180,000 exons and ~50 Mb or ~1% of the entire human genome. The captured patient DNA is then sequenced on a next-generation sequencer and an intensive bioinformatics analysis is performed to read the sequence (base calling), assess data quality, align sequences to a reference genome, call and annotate variants. Additional details regarding the technical aspects and limitations of exome sequencing have been described elsewhere.[12,14] An adequate 'depth' of coverage (e.g., redundancy with which each nucleotide is sequenced) is necessary to assure reliability and accuracy. Certain technical limitations can affect the analytical quality of exome data, and Sanger sequencing is often used to validate results and to eliminate false positives. Analysis of exome data to identify disease associated variants is aided greatly by knowledge of family structure and by collecting sequence data from first degree relatives who are either affected or unaffected (**Fig. 2**).

Whole genome sequencing may soon be sufficiently cost effective and time efficient to warrant its use as the mainstay in next-generation clinical genetic testing. Although the costs of sequencing an entire human genome are falling rapidly, there remain substantial challenges to data analysis that will slow widespread clinical implementation.[15] However,

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there is evidence that these challenges may be surmountable sooner than expected. A recent report demonstrated the feasibility of a rapid turnaround of genome sequencing results for determining the molecular diagnosis of a severe case of neonatal long-QT syndrome type 2 (*KCNH2* mutation) in a clinical setting.[16]

Classifying Variants and Interpreting Test Results

Genetic variants identified by clinical genetic testing laboratories are classified according to defined schema to enable a concise language for describing the best estimate of the clinical significance of a reported sequence variation (**Table 4**). In 2008, the American College of Medical Genetic (ACMG) Laboratory Quality Assurance Committee recommended six interpretative categories of sequence variants to standardize laboratory reporting of genetic test results.[17] Two of these categories are intended for variants with the strongest supporting evidence for pathogenicity ('Disease causing') or the lack of pathogenicity ('Not disease causing'). Other categories provide descriptors for variants with less certain clinical significance. A similar five-tiered scheme was proposed by the International Agency for Research on Cancer (IARC) to classify variants detected in subjects at-risk for hereditary cancer syndromes.[18] The IARC scheme additionally assigns quantitative probability ranges for each category. Commercial genetic testing laboratories may deploy additional derivatives of these classification schemes for standardized reporting. Importantly, evidence for or against pathogenicity of a given variant may evolve with new information and/or experimental data. Certain variants once deemed pathogenic may require reclassification based on new findings.[19,20]

Allele frequency in reference populations is typically used to distinguish common genetic variants from rare, candidate mutations. Resources for ascertaining population-based allele frequencies are listed in **Table 2**. The Single Nucleotide Polymorphism Database (dbSNP) is a freely available catalog of genetic variation within different species. Another resource, ClinVar, archives evidence-based reports of the relationships among genetic variation and phenotypes. The Human Gene Mutation Database (HGMD) maintains a collection of data on mutations in genes underlying or associated with human inherited disease.

More recently, databases of human genome and exome sequences have been curated to determine variant frequencies. The 1000 Genomes Project archives low coverage genome sequences on >1,000 individuals without disclosed phenotypic information.[21] Similarly, the Exome Sequencing Project funded by a Grand Opportunity (GO) grant from the National Institute of Heart, Lung and Blood Diseases (NHLBI) has populated the Exome Variant Server (EVS) with variant data deduced from exome sequencing of more than 6,500 individuals.[22] The Human Genetic Variation Browser [HGVD] database includes exome data obtained from 1208 Japanese subjects. These resources can be used to determine if a discovered variant is novel and therefore likely disease-causing by virtue of its absence or rarity in the general population. Certain variants with known association with specific genetic disorders may be captured by these large scale projects but the significance of these findings is uncertain. The presence of a genetic variant in a reference database does not necessarily exclude its potential pathogenicity, especially when the observed allele frequency is below the estimated population frequency of the disease. For example, the

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congenital long-QT syndrome has been estimated to affect 1 in 2500 live births based on a large scale neonatal ECG screening study coupled with candidate gene mutation discovery in nearly 45,000 Italian neonates.[23] By contrast, other variants reported as disease-associated in familial cardiomyopathy and certain arrhythmic disorders are present in reference databases at frequencies higher than expected based on the population prevalence of the respective phenotypes.[24-27] These findings suggest the need for caution in interpreting incidentally discovered variants without stringent criteria for assigning pathogenicity (e.g., segregation with phenotype in more than one family, functional evidence of deleterious consequences).

Variants of Unknown Clinical Significance

Results from clinical genetic testing may be confounded by the discovery of 'variants of unknown significance' (VUS) for which there is insufficient data to establish whether or not a particular variant predisposes to a disease. With the expanded use of exome/genome data in clinical medicine, interpreting VUS will become a larger challenge especially when variants in genes associated with monogenic disorders are incidentally discovered and reported. This problem is particularly vexing for most genetic disorders that have a high level of allelic heterogeneity and a preponderance of 'private' mutations. Additional evidence should be sought when possible to more firmly establish the relationship with disease risk in a family tested positive for a VUS. Segregation of the VUS among affected and unaffected family members may provide additional support for disease association, although this may be difficult to ascertain in small families, disorders with recessive inheritance or with incomplete penetrance. Laboratory research to establish whether a VUS has deleterious consequences may offer additional clues to pathogenicity but these are not standardized assays that yield results suitable for clinical decision making. Nonetheless, collaboration between clinicians and researchers provides an avenue to decrypt the growing burden of VUS.

There have been several computational strategies developed to help predict the potential effects of genetic variants on protein function in the research setting. Two of the more widely used methods are PolyPhen-2 and SIFT (Sorting Intolerant From Tolerant). SIFT uses protein sequence homology to assess the likelihood that a position-specific amino acid substitution will be damaging based on the premise that important residues will be conserved in the protein family throughout evolution.[28,29] SIFT was originally developed using prokaryotic gene mutation data, but was later tested on a large set of annotated human mutation data. PolyPhen-2 uses protein sequence-based and structure-based features to make predictions.[30] Another approach (Evolutionary Diagnosis [EvoD]) featuring statistical models based on evolutionarily weighted training data has been suggested to offer improved predictive power.[31] Newer, purportedly better methods have emerged recently,[32,33] but no particular algorithm appears superior to all others.[34] Disease specific models may have better performance.[35] Importantly, there have been few attempts to experimentally validate these *in silico* prediction models.[34,36]

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Managing Negative Results and Incidental Findings

Interpretation of a 'negative' genetic test in a symptomatic person is a significant challenge. A *true negative* result (e.g., technically successful, but no pathogenic findings) may occur because the test does not target the causative gene, possibly because a previously unknown genetic culprit is involved or the test panel was not comprehensive. Theoretically, this phenomenon will occur less frequently when exome sequencing is used as the testing platform. *False negative* results (e.g., no pathogenic findings reported even when one exists in the targeted gene) have other causes such as location of a mutation outside the region interrogated by the test[37] and existence of types of mutations (e.g., multi-exon deletion, duplication) missed by the most commonly used testing strategies that are designed for finding single nucleotide changes.[38-40] Repeat testing may sometimes overcome false negative results especially when there is a high level of clinical suspicion.[41]

The use of exome or genome sequencing may uncover medically actionable variants unrelated to the primary disorder that prompted the test. This point was illustrated by an examination of 1,000 participants randomly selected from the 6,500 subjects studied by the NHLBI Exome Sequencing Project.[42] In a survey of pathogenic variants in 114 genes selected because of associations with medically actionable genetic conditions, the frequency of highly penetrant and actionable variants was 1.2% for individuals of African descent and 3.4% for subjects with European ancestry. Among the pathogenic variants identified were several in genes associated with familial cardiomyopathy and congenital arrhythmia susceptibility. This demonstration of the prevalence of incidental findings in exome data in a research setting has prompted considerable debate regarding the best practice for reporting and managing such information.

Recently, the American College of Medical Genetics (ACMG) Working Group on Incidental Findings in Clinical Exome and Genome Sequencing recommended that laboratories performing clinical sequencing seek and report mutations in 57 genes including 30 genes responsible for cardiovascular phenotypes (**Table 5**).[43,44] These recommendations were designed to establish an initial reporting standard for laboratories engaged in exome/genome sequencing for genetic diagnosis. Inherent in the ethical framework within which these recommendations were based was the assumption that the ordering clinician would bear responsibility for obtaining informed consent from the patient including pretest and posttest genetic counseling about the potential risks and benefits of testing.[45] The ACMG recommendations have been challenged both on scientific and ethical grounds,[46,47] but defended by emphasizing that incidental findings provide an opportunity for patient education and collaboration between patient and provider to define the best course of action.[48] A recent amendment to these recommendations by ACMG suggests that patients should be given an opportunity to 'opt out' of the analysis of medically actionable genetic variants at the time samples are sent for initial testing.[49] Genetic counselors may have special value in helping patients make such decisions.

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Pharmacogenomic Profiling

Clinical genetic testing can be applied to reveal genomic variants associated with inter-individual differences in drug responses (e.g., variable therapeutic efficacy or adverse effects). The term *pharmacogenetics* was originally coined as the study of unusual drug response traits exhibiting Mendelian inheritance in families (e.g., glucose-6-phosphate dehydrogenase deficiency [*G6PD*, Xq28], pseudocholinesterase deficiency [*CHE1*, 3q25], malignant hyperthermia susceptibility [*RYR1*, 19q13]). By contrast, *pharmacogenomics* has been used to describe mainly population-based studies defining genes or loci associated with differences in drug responses among groups of unrelated individuals. Drug response variability is often explainable by differences in either pharmacokinetics (e.g., drug metabolism for biotransformation or elimination) or pharmacodynamics (e.g., response of the target molecule). More than 125 FDA-approved drugs have pharmacogenomic information in their labeling including some with 'boxed warnings' advising physicians to acquire specific genomic data on patients for whom the drug is being considered. The Clinical Pharmacogenetics Implementation Consortium (CPIC) formed by the NIH-funded Pharmacogenomics Research Network[50] has developed a series of evidence-based, consensus guidelines to enable the translation of clinical genetic test results into actionable prescribing decisions for specific drugs.[51]

The emergence of pharmacogenomics has offered new opportunities for achieving the goal of personalized medicine by utilizing clinical testing for medically actionable variants including drugs commonly prescribed for cardiovascular disorders.[52-54] Genetic testing may have value in predicting efficacy of specific drug therapy (e.g., *CYP2C19* genotyping in the setting of antiplatelet therapy with clopidogrel),[55] identifying persons what are at risk for specific adverse reactions (e.g., *SLCO1B1* genotyping to assess risk of simvastatin-induced muscle toxicity),[56,57] or for determining initial dosage (*VKORC1* and *CYP2C9* genotyping for warfarin dosing).[58] Strategies for implementing pharmacogenomic testing in clinical settings have either adopted a one gene at a time approach[59,60] or advocated for preemptive testing of multiple variants.[61-63] Decision support is essential for educating providers about interpretation of test results and for presenting specific prescribing actions. Physician adoption and utilization of pharmacogenomics testing can be high in settings where point-of-care decision support is provided,[64] but somewhat less effective when results are merely faxed to providers several days later.[65] Further research including randomized clinical trials, such as those recently reported for oral anticoagulants,[66-68] are needed to determine value of pharmacogenomic testing in clinical practice.

Testing for Complex Genetic Traits

Whereas genetic diagnostics have become routine and standard-of-care for many monogenic disorders, laboratory assessments of inherited risk for more common and genetically complex diseases are seldom performed in medical practice.[69] During the past decade, genome-wide association studies (GWAS) have mapped more than a thousand disease susceptibility loci based on the "common disease-common variant" hypothesis that posits a major portion of risk for a common disease in populations is conferred by a limited number of common genetic variants.[70] A frequent observation made by GWAS is that common

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variants account for only a small proportion of population attributable risk (estimated by an odds ratio with reported values often less than 1.5), and results from these population-based genomic studies have been difficult to translate into risk predictions for individuals.

The Center for Disease Control (CDC) has recently launched the Evaluation of Genomic Applications in Practice and Prevention (EGAPP) initiative to establish and evaluate an evidence-based process for assessing applications of genomic technologies in the clinical setting.[71] Using this systematic approach, an evaluation of specific cardiovascular disease genomic risk variants in 29 candidate genes found only weak to moderate evidence supporting clinical validity. A possible exception was with the association of 9p21 variants with heart disease, which seemed more robust although the estimated additional benefit of knowing genotype at this locus was judged as negligible.[72,73] Further studies including clinical trials are needed to determine the impact of genomic risk testing on clinical outcomes, physician practice, patient behavior and health care costs.

Despite the uncertain benefits to individuals undergoing genomic risk profiling, a few for-profit business ventures have capitalized on direct-to-consumer marketing of genomic testing. The leader in this emerging industry has been 23 and Me, Inc. (Mountain View, CA). Such companies market laboratory genetic testing along with interpretive services for assessing genomic risk for specific diseases, pharmacogenomic profiling and analysis of ancestry directly to consumers without requiring input from healthcare providers.[74] The intent of these services is to empower individuals with personal genomic information that might help improve health through lifestyle changes and more informed medical decision-making. Collections of population-based genomic data also has enabled research opportunities.[75-77] Initial research on the impact of direct-to-consumer genomic testing suggests there is little harm or benefit.[78,79]

The advent of direct-to-consumer genetic testing ignited considerable debate related to the medical value of the genomic risk discoveries on which these proprietary tests are based and on the accuracy of the data provided to consumers.[74,80] Unlike other genetic diagnostics, which are classified as medical devices requiring FDA approval, direct-to-consumer tests have not been subject to regulation. In late 2013, following the launch of an aggressive campaign to sell its product, the FDA ordered 23 and Me to discontinue marketing its primary genomic profiling service.[81,82] New regulatory regimes stimulated by these actions could impact other emerging genomic testing paradigms including whole-genome sequencing.

Summary

Technical advances have ushered in a new era of genetically-informed diagnosis that will impact both rare and common cardiovascular disorders. Understanding the capabilities and limitations of genetic testing in various clinical settings is vital to effectively translate the tsunami of medical breakthroughs generated during this genomic era into clinical practice. Accomplishing these goals will require changes in medical education, health care delivery systems, medical informatics, and more informed patients and providers.

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Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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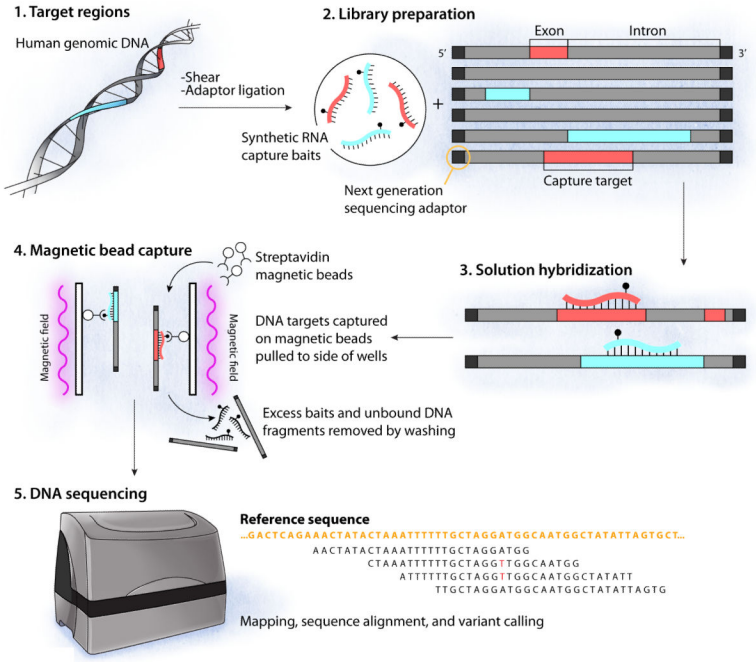

Figure 1.

Illustration of steps involved in DNA capture and next-generation sequencing (adapted by permission from Macmillan Publishers Ltd: *Nature Genetics Reviews*, Bamshad, et al., 2011).[12] Intact human genomic DNA is sheared into random, small fragments then synthetic adaptor sequences are added to the fragment ends. Next, the pool of adaptor-ligated DNA fragments are incubated with a complex mixture of biotinylated RNA 'baits' designed to hybridize to all coding exons by complementary nucleic acid base pairing. Following hybridization, targeted DNA regions are captured using streptavidin coated magnetic beads, which selectively bind the biotinylated 'bait' strands and simultaneously immobilize any bound DNA fragments. After washing to remove excess bait and unbound DNA, a library of captured DNA is prepared for sequencing. Finally, all captured DNA is sequenced a several fold redundancy using a next-generation sequencer and the data output are analyzed.

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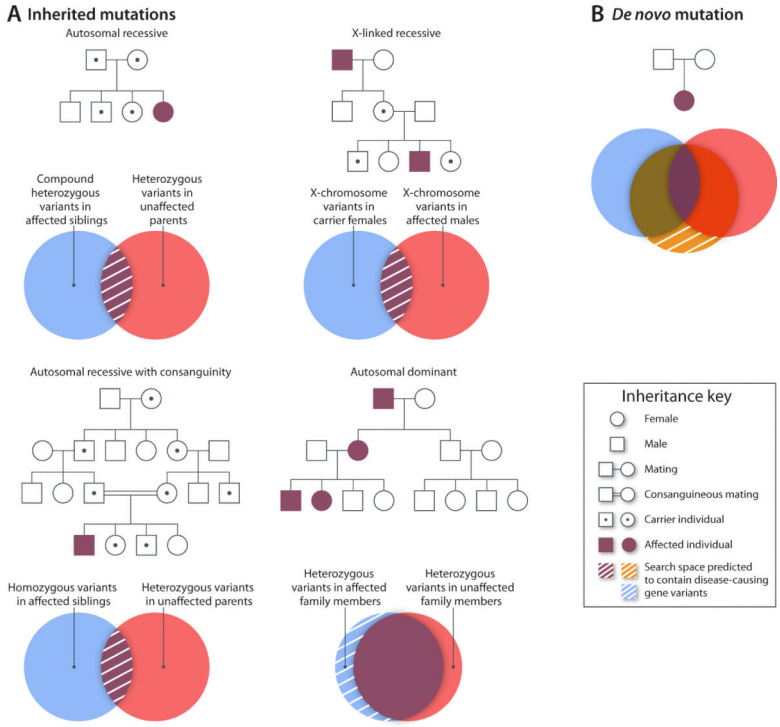

Figure 2.

Approaches for gene discovery using next-generation sequencing data (adapted by permission from Macmillan Publishers Ltd: *Nature Genetics Reviews*, Boycott, et al., 2013).[83] Pedigrees illustrating various inheritance models are presented in which symbols represent males (squares), females (circles), affected (shaded), unaffected (unshaded) and obligatory mutation carriers (symbols with a central dot). (**A**) Mendelian inheritance patterns (autosomal recessive, autosomal dominant and X-linked) are illustrated. (**B**) Pedigree illustrating a parent-child trio in which a *de novo* (e.g., non-inherited) mutation is present in the affected child. Venn diagrams beneath each representative pedigree indicate how variants can be shared among family members. The hatched regions within these diagrams denote the search space (e.g., regions of overlap or non-overlap) predicted to contain disease-causing gene variants.

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Table 1

Uses of Genetic Testing in Cardiovascular Disorders

Pre-implantation testing: Performed on human embryos derived by *in vitro* fertilization to guide selection of unaffected embryos for implantation.
Diagnostic testing: Performed at any age to diagnose specific genetic disorders.
Carrier testing: Performed on individuals with a family history of a genetic disorder to guide reproductive counseling about the risks of having an affected child.
Presymptomatic testing: Performed individuals who are at-risk for a genetic disorder at an age before the disorder typically develops. This is also called predictive testing.
Postmortem testing: Performed on deceased individuals as part of an evaluation for the cause of death especially when sudden cardiac death was evident.
Pharmacogenomic testing: Performed to determine risk for unfavorable or adverse effects of drug therapy, or to determine the most appropriate dose.
Risk prediction testing: Testing genomic loci with known population-based disease associations to assess risk for the condition in an individual.
Direct-to-consumer testing: Commercial genetic testing offered to individuals without involvement of a physician. This type of testing is not designed to make specific genetic diagnoses.

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Table 2

Internet resources and databases

Genetic Testing Resources
GeneTests™
http://www.genetests.org/
Genetic Testing Registry
http:/www.ncbi.nlm.nih.gov/gtr/
GeneReviews®
http://www.ncbi.nlm.nih.gov/books/NBK1116/
United Kingdom Gene Testing Network
http://ukgtn.nhs.uk/
Genetic Variant Databases
Single Nucleotide Polymorphism Database (dbSNP)
http://www.ncbi.nlm.nih.gov/projects/SNP/
NIH ClinVar
https://www.ncbi.nlm.nih.gov/clinvar/
The Human Gene Mutation Database (HGMD)
http://www.hgmd.cf.ac.uk/ac/
1000 Genomes Project
http://www.1000genomes.org/
Exome Variant Server
http://evs.gs.washington.edu/EVS/
Human Genetic Variation Browser (HGVD)
http://www.genome.med.kyoto-u.ac.jp/SnpDB
Pharmacogenomic Resources
Pharmacogenomics Knowledgebase (PharmGKB)
https://www.pharmgkb.org/
Clinical Pharmacogenetics Implementation Consortium (CPIC)
http://www.pharmgkb.org/page/cpic

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Table 3

Genetic disorders affecting the cardiovascular system.

Cardiomyopathies
Hypertrophic cardiomyopathy
Familial dilated cardiomyopathy
Arrhythmogenic cardiomyopathy
Restrictive cardiomyopathy
Left ventricular noncompaction
Arrhythmia susceptibility
Congenital long QT syndrome
Brugada syndrome
Catecholamineric polymorphic ventricular tachycardia
Atrial fibrillation
Short QT syndrome
Disorders of lipid metabolism
Familial hypercholesterolemia
Vascular disorders
Primary pulmonary hypertension
Hereditary hemorrhagic telangiectasia
Disorders of blood pressure regulation
Liddle syndrome
Glucocorticoid remediable aldosteronism
Bartter syndrome
Gitelman syndrome
Pseudohypoaldosteronism
Congenital heart malformations
Multi-system and developmental disorders with cardiovascular manifestations
Marfan syndrome
Duchenne muscular dystrophy
Noonan syndrome
LEOPARD syndrome
Holt-Oram syndrome
CHARGE syndrome

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Table 4

Classification of Genetic Variant Pathogenicity.

American College of Medical Genetics [17]

- **Disease causing**

  Sequence variation has been reported previously and is a recognized cause of the disorder

- **Likely disease causing**

  Sequence variation has not been reported previously but is a type (e.g., nonsense mutation, frameshift) expected to cause the disorder

- **Possibly disease causing**

  Sequence variation has not been reported previously and is a type that may or may not be causative of the disorder (e.g., nonsynonymous variant)

- **Likely not disease causing**

  Sequence variation has not been reported previously and is probably not causative of the disease

- **Not disease causing**

  Sequence variation has previously been reported and is a recognized neutral (e.g., benign) variant

- **Variant of unknown clinical significance (VUS)**

  Sequence variation is not known or expected to be causative of disease but is associated with a clinical presentation

International Agency for Research on Cancer[18]

- **Definitely pathogenic**

  >0.99 probability of being pathogenic

- **Likely Pathogenic**

  0.95-0.99 probability of being pathogenic

- **Uncertain**

  0.05-0.949 probability of being pathogenic

- **Likely not pathogenic**

  0.001-0.049 probability of being pathogenic

- **Not pathogenic**

  <0.001 probability of being pathogenic

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Table 5

Genes involved with cardiovascular disorders recommended for automatic reporting of incidental variants from exome and genome testing.

Phenotype	Gene	Variants to Report*
Vascular disorders		
	FBN1	KP, EP
	TGFBR1	KP, EP
Marfan syndrome, Loeys-Dietz syndrome, familial aortic aneurysm	*TGFBR2*	KP, EP
	SMAD3	KP, EP
	ACTA2	KP, EP
	MYLK	KP, EP
	MYH11	KP, EP
Cardiomyopathies		
	MYBPC3	KP, EP
	MYH7	KP
	TNNT2	KP, EP
	TNNI3	KP
	TPM1	KP
Hypertrophic cardiomyopathy, dilated cardiomyopathy	*MYL3*	KP
	ACTC1	KP
	PRKAG2	KP
	GLA	KP, EP
	MYL2	KP
	LMNA	KP, EP
Arrhythmic disorders		
Catecholaminergic polymorphic ventricular tachycardia	*RYR2*	KP
	PKP2	KP, EP
	DSP	KP, EP
Arrythmogenic cardiomyopathy	*DSC2*	KP, EP
	TMEM43	KP
	DSG2	KP, EP
	KCNQ1	KP, EP
LQTS, Brugada syndrome	*KCNH2*	KP, EP
	SCN5A	KP, EP
Dyslipidemias		
	LDLR	KP, EP
Familial hypercholesterolemia	*APOB*	KP
	PCSK9	KP

*KP, known pathogenic (previously reported and is a recognized cause of the disease); EP, expected pathogenic (unreported variant but of a type such as truncating that is expected to cause the disorder).