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CURRENT OPINION

The current role of next-generation DNA sequencing in routine care of patients with hereditary cardiovascular conditions: a viewpoint paper of the European Society of Cardiology working group on myocardial and pericardial diseases and members of the European Society of Human Genetics

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Introduction

Cardiomyopathies, arrhythmic syndromes, aortopathies, and other cardiovascular diseases with Mendelian inheritance are relatively frequent conditions for which genetic testing is recommended in various guidelines.[1,2] The most widely recognized indication for genetic testing in patients with these conditions is to identify a causative mutation and subsequently provide pre-symptomatic or predictive testing of relatives who are at risk of developing the same disease at a later stage. This process of cascade screening of family members ensures adequate clinical surveillance of mutation carriers and allows non-carriers to be discharged from clinical follow-up. A number of studies have reported a greater cost-effectiveness combining molecular screening with clinical screening compared with isolated clinical investigations.[3]

Previously, genetic testing was based on conventional techniques like Sanger sequencing analysing genes one by one, but recent advances in DNA sequencing technologies have made it possible to investigate large numbers of disease genes simultaneously, making mutation analysis much faster and cheaper. These new methods are known as next-generation sequencing (NGS) and represent a major advance in the ability to identify causative mutations in families affected by genetic diseases (see Supplementary material online, *Figure S1*).[4,5] However, analysis of large numbers of genes may identify a number of sequence variants of uncertain clinical significance (VUS). As a result, cardiologists and clinical geneticists who counsel and manage families with inherited cardiovascular disorders are facing a major challenge in determining the clinical relevance of NGS results.[6,7]

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This paper gives a brief overview of the principles of NGS, discusses the general strategies for the interpretation of sequencing results, and reviews the implications of NGS for cardio-genetic services. In addition, issues related to genetic counselling and ethical considerations are discussed. A summary of viewpoints is given in *Table 1*.

Methods for identification of disease-causing mutations

Until recently, mutation analysis has been performed mostly using Sanger sequencing in which the order of nucleotides in the coding sequences of a gene (exons) is analysed in series one after the other. The method is accurate and reproducible but also labour intensive and expensive, which has limited testing to analyses of relatively few disease genes.

NGS uses a highly parallelized sequencing process, which makes it possible to investigate large numbers of genes simultaneously at greater speed and at lower cost than by Sanger sequencing (see Supplementary material online, *Figure S1*).[4,5] The method is particularly useful in the context of hereditary cardiovascular conditions, since the number of underlying disease genes and causative mutations is significant for most of the disorders. In addition, the size of some of the disease genes—most notably Titin (*TTN*) and the Ryanodine Receptor (*RyR2*)—impedes the use of Sanger sequencing as an analysis tool in clinical practice.

NGS technology can be used in different ways. Many diagnostic laboratories offer 'targeted gene panels', in which there is a focus on a set of genes known to be associated with specific disorders. Others provide whole exome sequencing (WES) which covers almost all protein-coding sequences or whole-genome sequencing (WGS) that includes nearly all non-coding sequences as well.

Table 1 Summary of viewpoints on the use of next-generation sequencing in genetic diagnosis of hereditary cardiovascular conditions

- Before genetic testing it is important to inform the patient about the challenges in interpretation of sequencing results of multiple genes and discuss the implications of unsolicited findings
- In a clinical diagnostic setting only recognized disease genes should be investigated in patients fulfilling diagnostic criteria of a specific cardiovascular condition
- Whole exome/genome sequencing is considered to be a diagnostic method in development and should be used for genetic diagnosis only if filtered against recognised disease genes. The coverage should allow identification of all exomic variants in these genes
- Interpretation of sequencing results should take place in close collaboration between bio-informaticians, cardiologists, molecular biologists, clinical geneticists, preferably in expert centres
- Development of public databases worldwide with clinical information and sequencing results are essential to ensure optimal patient management
- Prospective re-evaluation of variants of uncertain significance is essential
- Algorithms for re-contacting referring physicians and patients have to be developed

Targeted gene panels have been shown to generate results with analytical quality identical to Sanger sequencing and have the advantage of being faster and cheaper with a better coverage and sensitivity than WES and WGS.[4] Although WES and WGS make it possible to perform an unbiased search for mutations in all human genes, this approach is currently considered less appropriate for routine diagnostic purposes as not every part of the coding sequence is sufficiently covered, which may lead to false-negative results. In addition, issues related to management of the huge amount of data generated by WES and WGS remain to be solved before these approaches are suitable for routine use in a clinical setting.[8,9]

Interpretation of sequencing results

With the introduction of NGS into clinical practice, the number and size of genes investigated have increased dramatically. Since the number of variants identified by NGS is almost proportional to the total number of DNA bases sequenced, many more variants are being identified and need to be classified as pathogenic, benign, or VUS.[10,11] In order to make such distinctions, a rigorous process of interpretation is necessary to avoid misclassification and thereby ensure correct counselling. This relies on a number of complementary investigations.

(1) *Frequency of variants in healthy controls*
Several public databases provide information about the frequency of variants within the coding sequence of the human genome based on WES and WGS of thousands of apparently healthy controls. Once a sequence variant has been identified in a patient, it is important to determine whether it is present or absent in such databases. A high-frequency among controls indicates that the variant identified is likely to represent normal variation while a very low frequency or complete absence suggests a potential disease-associated mutation.

(2) *Published data*
It is important to clarify whether the variant has already been reported as disease causing. However, the evidence for causation needs careful evaluation since much of the published data that were generated in the pre-NGS era involved a limited number of controls. It has become evident following the introduction of NGS that a significant number of rare variants previously reported to be pathogenic are in fact likely to be benign due to their presence in the general population.[12]

(3) *Co-segregation in families*
Co-segregation of a variant with the condition in a large family with many affected individuals usually provides strong evidence for causation. However, families with multiple affected individuals is a rare occurrence and the most common clinical scenario is that of a novel sequence variant in an individual with only few or no other clinically affected relatives. Consequently, many novel variants identified by NGS will be classified as VUS and thereby represent an inconclusive test result at present.

(4) *Likely effect on the transcribed protein and evolutionary conservation*
Sequence variants exert different effects on protein structure that may or may not be pathogenic. The probable impact on protein function can be estimated from the type of mutation

(nonsense, missense, splice site), the level of conservation through evolution by comparison to DNA-sequences of other species, and by using *in silico* prediction tools. These analyses provide information about the likelihood of pathogenicity, but cannot be used in isolation to classify a sequence variant as relevant for clinical decision-making or genetic counselling.

Informed consent and ethical considerations

The ability to sequence all of the human genome and the consequent identification of many VUS presents new challenges for the counselling of individuals before and after genetic testing. Particular issues to consider are the possibility of reclassification of genetic variants from benign to disease causing (or *vice versa*) at a later date due to the generation of new data. In addition, unsolicited findings in other genes such as recognized cancer genes are reported in 1–3% of patients undergoing WES or WGS.[10] It is therefore essential to discuss with patients prior to WES or WGS whether genes unrelated to their condition should be interpreted and whether they would like to be informed about potentially relevant findings in genes unrelated to the condition for which they are being tested. Findings in such 'actionable genes' may potentially influence the future health condition of the patient and their family and thereby indicate regular follow-up to ensure timely treatment and genetic counselling.

The ethical concerns about incidental findings and the current technical limitations of NGS with respect to gene coverage mean that, for the present, WES and WGS are considered to be a diagnostic method in development.[8] We suggest that only recognized disease genes with substantial evidence of causality should be investigated when offering routine genetic testing. Other genes with less evidence of causality should be classified as candidate genes and primarily investigated for research purposes.

Sharing information in public databases

To take the full advantage of NGS, it is important to develop dedicated databases that combine sequencing data and clinical information about patients in order to share, compare, and continuously update knowledge for medical use. This will facilitate correct interpretation of identified sequence variants, ensure clinical efficiency, and maintain on-going evaluation of reported sequencing data.[13]

In contrast, if this information is not shared, there is a considerable risk of misinterpretation of the impact of the variants identified, which may lead to wrongful counselling of affected families.

Complexities in diagnosis and organisation of cardio-genetic services

The development of NGS has not only made it feasible to offer genetic testing in large numbers of genes and to more patients when compared with previous methods, but it has also accelerated the pace of new disease gene discovery. The result is an ever growing list of candidate genes in screening protocols and greater complexity in the interpretation of genetic variants both of which make it necessary to develop continuing education of molecular biologists in bioinformatics and clinicians caring for patients with hereditary cardiovascular conditions.

The increased availability and ever decreasing cost of NGS make it tempting to apply less stringent indications for genetic testing. However, it is well established that the diagnostic yield of genetic investigations is highest in patients with familial disease who fulfil diagnostic criteria for the condition under investigation. In patients with an ambiguous clinical diagnosis, it is often very difficult to establish whether a specific sequence variant is disease causing and usually requires careful clinical assessment of patients and their relatives.

Therefore, to ensure accurate clinical diagnosis, provide optimal counselling and management of families with hereditary cardiovascular conditions it is essential that cardiologists, molecular biologists, bio-informaticians, and clinical geneticists, work closely together as a team, ideally in expert centres. This facilitates multidisciplinary case discussion and constant review of the indications for genetic testing, counselling strategies, and interpretation of sequencing results. By pooling the experience of such teams in public databases it will soon be possible to translate all the data generated by NGS into usable knowledge for the benefit of patients and their families.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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