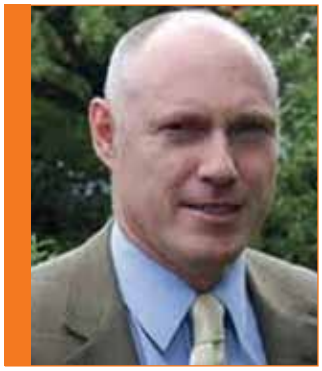
Rapid DNA mutational profiling for Familial Hypercholesterolemia

Since the mapping of the human genome was completed over a decade ago, our knowledge of genetic drivers of disease continues to evolve at an ever-quickening pace. Consequently, genetic testing and pharmacogenomics have become common within the healthcare system and have generated the knowledge that has empowered us to both understand and influence our lifelong health through pre-emptive intervention.

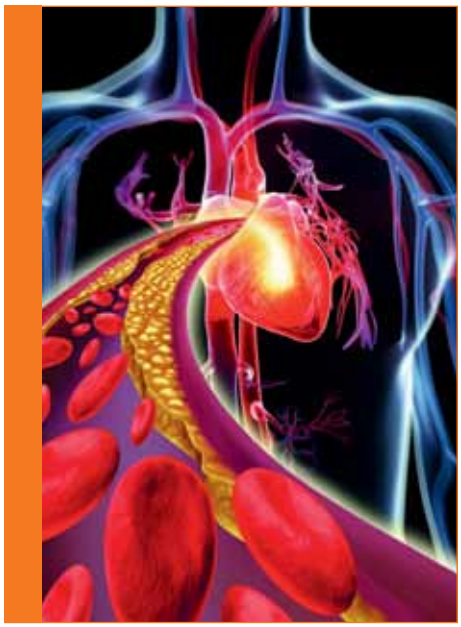
Progress in medical genomics and its impact on healthcare cannot be understated; from genotyping patients to predict drug response, to stratifying patients according to the risk of a disease, molecular testing is having a very positive impact on many patient treatment pathways. Undoubtedly, we are now more aware and in control of our health than ever before. It is no surprise then that the molecular diagnostic market has become the fastest growing segment of the IVD industry with assays serving the gamut of disease areas and breaking new boundaries in personalized healthcare. Despite the public appetite and availability of powerful molecular diagnostic assays that can unequivocally diagnose genetic disorders, their use has not gained universal acceptance. Many traditional diagnostic tests continue to under-diagnose, or diagnostic testing is not attempted, leading to missed opportunities for early and appropriate therapy intervention of potentially life-threatening diseases. One prime example where a molecular diagnostic approach can improve health is mutation profiling for Familial Hypercholesterolemia (FH).



Martin Crockard, PhD, Molecular Diagnostics Manager for Randox Laboratories. Dr. Crockard works closely with the company's engineering, regulatory affairs, marketing and manufacturing departments to develop both multiplex arrays and complementary analysers.

Familial Hypercholesterolemia

Familial Hypercholesterolemia (FH) is a genetic disorder of lipoprotein metabolism. It is a common autosomal dominant, or inherited, disease which affects the plasma clearance of LDL-cholesterol (LDL-C), resulting in premature onset of cardiovascular disease (CVD) and a higher mortality risk.

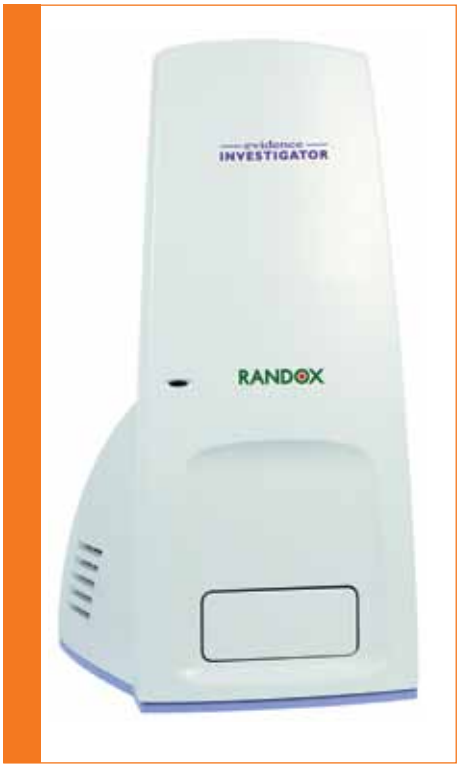


Early diagnosis of FH is very advantageous as by the time heterozygous FH sufferers enter early adulthood they will have accumulated years of continuous build-up of fatty or lipid masses in arterial walls and are at one hundred-fold greater risk of a heart attack than their non-FH peers. If left untreated, men and women with heterozygous FH with total cholesterol levels of 8–15 mmol/L typically develop coronary heart disease (CHD) before age 55 and 60, while homozygotes with total cholesterol levels of 12–30 mmol/L typically develop CHD very early in life and if untreated die before age 20.

Clinical diagnosis of FH relies on five criteria: family history, clinical history of premature CHD, physical examination for xanthomas and corneal arcus, very high LDL cholesterol on repeated measurements, and / or a causative mutation detected by molecular genetics. To formally quantify this, a number of sets of statistically and genetically validated criteria have been devised; namely the Dutch Lipid Clinic Network Criteria and the Simon Broome Criteria. These classify suspected cases into definite, possible and probable diagnoses of FH. In the absence of definitive diagnosis through detection of a causative mutation using molecular genetics, clinical diagnosis could miss a considerable proportion of FH patients, particularly those with a mild phenotype and the pediatric population in whom the phenotype has not appeared yet.

The UK, US and international guidelines now recommend that probable or possible FH patients undergo a DNA test to confirm the diagnosis of FH. Recommendations also advocate that once an activating mutation has been found in one family member (the index case), cascade screening of that mutation in first degree relatives of the index case should proceed. Cascade screening using a molecular assay can thus identify index family members who may otherwise be asymptomatic.

The good news is that if detected early, FH can be treated successfully with lipid lowering therapy and lifestyle changes. In comparison to other hyperlipidemias, FH therapy tends to be more aggressive, so definitive diagnosis has additional benefits in determining care packages. Statin drug therapy significantly reduces the morbidity and mortality from premature



Investigator

coronary disease in FH, particularly if affected individuals are identified and treated in childhood or early adulthood. Accurate and early diagnosis of specific mutations can result in a better overall outcome for patients through the prescribing of tailored treatments to reduce morbidity and mortality from premature cardiovascular disease. Different mutations can dictate different directions of management, such as the poorer response to lipid-lowering therapy with certain LDLR mutations. The identity of the gene involved can potentially aid the clinician to decide on how aggressive the treatment strategy will be.

Mutation diagnosis also provides clarity, and can help with an individual's understanding and acceptance of their condition. Also a greater compliance with cholesterol lowering medication is observed with those who have been genetically diagnosed with FH.

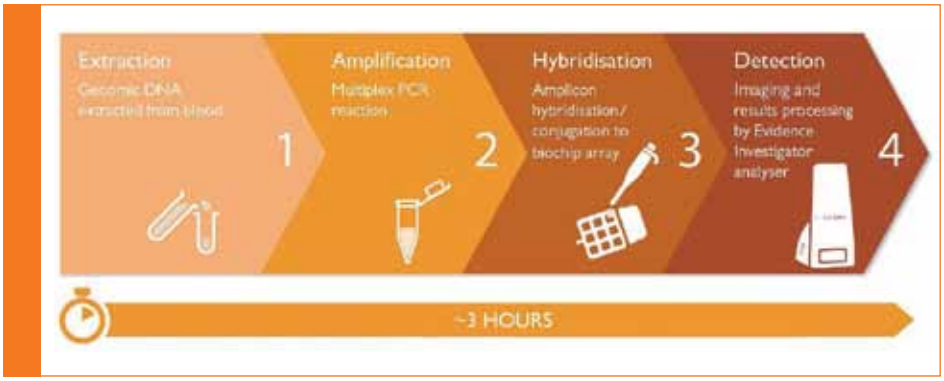
Mutational profiling of FH

Currently, ~1200 mutations have been documented worldwide in LDLR; these affect all functional domains of the LDL receptor protein and include single-nucleotide mutations, copy number variations, and splicing mutations throughout the LDLR gene. A single mutation, Arg3500Gln, is the only common FH-related mutation in APOB, while c.1120G>T mutation is predominately detected in PCSK9. Heterozygous LDLR, APOB, and PCSK9 mutations are found in >90%, ~5%, and ~1%, respectively, of heterozygous FH subjects with a causative mutation. Prevalence varies geographically.

The abundance of different FH mutations can make genetic testing labour-intensive and costly, with many laboratories defaulting to performing expensive and lengthy Next Generation Sequencing (NGS) tests in an effort to ensure a comprehensive mutational screen. However, as our understanding of the genetic drivers of FH, as well as common population-specific mutations, increases, novel assays and techniques are being developed to meet the needs facing clinical genetics laboratories, including cost, throughput and time to result.

Randox Laboratories have developed The Familial Hypercholesterolaemia (FH) Arrays I and II that are rapid, simple and accurate diagnostic tests which enable simultaneous detection of 40 FH-causing mutations (20 mutations per array) within the LDLR, ApoB and PCSK9 genes. The assay is based on multiplex PCR followed by biochip array hybridization. Using mutation rate data from a study of 500 UK and Ireland families with genetically-confirmed FH, the Randox FH Arrays are capable of detecting approximately 71% of activating mutations in this population. The mutations will also be detected in other geographical regions.

The assay can be completed from extracted DNA to an easy-to-interpret result report in 3 hours, with the requirement for only 20ng of genomic DNA per array. The system can be used to detect small base changes, insertions and deletions within the same multiplex PCR, allowing addition of new FH mutational targets if required. The arrays are designed for use on the Evidence Investigator (Randox Laboratories Limited, Crumlin, UK). This instrument has been developed alongside Randox's proprietary Biochip Array Technology (BAT), a multiplex testing platform founded on ELISA principles that currently has application within clinical immunoassays, drug development R&D, clinical research, forensic and clinical toxicology, veterinary drug residues and molecular diagnostics.



FH Array I and II workflow

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Randox's multiplex assays, such as FH Array I and II, have been specifically designed to detect the most common mutations, provide a cost-effective and clinically relevant alternative to NGS testing. Targeting the most commonly detected mutations in a given population enables diagnosis within hours rather than months. Where a mutation is identified in an index patient, cascade testing of family members only requires the mutation in question to be targeted; therefore negating the use of broad profiling approaches such as NGS in this setting.

Conclusion

FH is a common yet underdiagnosed condition that poses a significant risk to public health worldwide. In 2008, cardiovascular diseases were the leading cause of non-communicable deaths worldwide, with an estimated mortality rate of 17 million people. Raised cholesterol was attributed to 2.6 million deaths. Understanding a person's genetic predisposition to cardiovascular disease through genetic testing will allow patients to receive appropriate therapeutic and interventional treatment to reduce morbidity and mortality associated with cardiovascular disease.

Pioneering multiplex diagnostic assays, tailored to incorporate the relevant FH-causing mutations, provide a promising future for both genetic laboratories, where a rapid, cost-effective approach to determine mutational status in cases of suspected FH is enabled, and the patient, whose treatment and care pathway is managed effectively.

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