Opportunities and challenges in using targeted next–generation sequencing for the diagnosis of dyslipidemias

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Abstract

Dyslipidemias are disorders in lipid metabolism that can jeopardize one's metabolic and cardiovascular health. Historically, dyslipidemias were diagnosed using biochemical findings such as abnormal levels of lipoproteins in the blood. Development of many cases of dyslipidemias are due to underlying genetic factors, but older methods of DNA sequencing were too slow and costly to be practically used in the clinic. Targeted next–generation sequencing (NGS) offers a unique opportunity to determine genetic diagnoses of inherited dyslipidemias more efficiently and at lower costs compared to older methods. NGS approaches to diagnosing dyslipidemias have been validated in several studies demonstrating the ability to create gene panels that can accurately diagnose patients that in some cases went undetected by other clinical guidelines. Advancements in NGS technologies provide new opportunities for the routine incorporation of a patient's genetic information into clinical care for dyslipidemias.

Disorders of lipid metabolism, otherwise known as dyslipidemias, are among the strongest risk factors for atherosclerosis and coronary artery disease (CAD).[1] About 2.4 million Canadians have CAD, and it is a leading cause of death in Canada.[2,3] Lipids are transported in the body inside lipoprotein particles that exist in various forms in the body such as low–density lipoproteins (LDL) and high–density lipoproteins (HDL). Each of these particles serves a distinct role in the body, and dysregulation of these lipoproteins can result in a number of clinical manifestations. For example, familial hypercholesterolemia (FH) is one of the most well–studied dyslipidemias and results in abnormally high LDL–C levels that can contribute to development of atherosclerosis and premature cardiovascular disease.[4] FH is an autosomal dominant trait that is typically caused by a heterozygous mutation in the LDLR, APOB, or PCSK9 genes and is just one example of numerous types of dyslipidemias that have a clear genetic basis (Table 1).[5] Therefore, being able to readily obtain genetic information about a patient can improve the diagnosis and management of dyslipidemias. Next–generation sequencing (NGS) is a broad term that encompasses DNA sequencing technologies that have emerged post–Sanger sequencing; while a technical comparison of sequencing technologies is beyond the scope of this review, Sanger sequencing is the original method of DNA sequencing and is limited by the fact that it can only analyze a single DNA fragment at a time. On the other hand, NGS can analyze multiple DNA fragments in parallel with exponentially more data being produced at a fraction of the time and cost.[6] While both methods still serve some purpose in clinical research today, for most purposes NGS is thought to have much greater potential for offering the ability to incorporate genetic information into routine clinical care.[7] Targeted NGS is a type of sequencing where one determines the presence of variants in a predetermined set of genes, as opposed to sequencing the whole genome, and it is easier to sift through the data for clinically actionable results. It also provides greater power to detect rare variants in relevant genes.[8] This commentary will discuss the recent advancements in using targeted NGS for diagnosing dyslipidemias and considerations for the implementation of this technology in clinical care.

Currently, the diagnosis of dyslipidemias is based primarily on clinical and biochemical findings, such as abnormal LDL, HDL, or triglyceride (TG) levels in the plasma, family history, and physical examination.[9] Management of dyslipidemias involves both pharmacological and behavioural interventions, depending on the risk level of the patient.[10] For example, guidelines published by the Canadian Cardiovascular Society (CCS) in 2016 recommend that patients with LDL–C >5 mmol/L and an additional risk factor such as atherosclerosis receive statin drug therapy.[10] Previous studies have demonstrated that genetic variations in specific genes (Table 2) are closely associated with abnormal lipid levels, but a patient's risk level in many guidelines is determined using metrics that do not take into account genetic factors, such as the Framingham Risk Score.[9,11] If genetic information was made more readily available to clinicians through NGS, guidelines may be updated with genetic criteria that may better assess a patient's risk level. For example, studies have demonstrated that calculating a genetic risk score using variants identified in previous genome–wide association studies can be used as an independent predictor for development of cardiovascular disease.[12] DNA sequencing is already being used to supplement biochemical methods of diagnosis of certain dyslipidemias such as chylomicronemia syndrome.[9,13] Johansen et al.[14] and Sadananda et al.[15] have both used

Table 1 | Select dyslipidemias and associated clinical features.

Dyslipidemia	Common Genetic Basis	Clinical Features
Familial hypercholesterolemia (FH)	Autosomal dominant mutations in LDLR, APOB, or PCSK9 genes	Xanthomas, arcus corneae, premature CAD
Familial defective apo B-100	Autosomal dominant missense mutation in apoB-100	Xanthomas, arcus corneae, premature CAD
Tangier disease	Autosomal recessive mutation in gene coding for ABCA1	Peripheral neuropathy, hepatosplenomegaly
Apolipoprotein A1 deficiency	Mutations in APOA1 gene	Atypical xanthomas, corneal opacification, premature CAD
LCAT deficiency	Autosomal recessive mutations in LCAT gene	Corneal opacification, hepatosplenomegaly
Chylomicronemia syndrome	Mutations in gene coding for LpL	Failure to thrive, anorexia, nausea, vomiting, pancreatitis

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Table 2 | Example monogenic dyslipidemia phenotype and associated genes.

Phenotype	Gene	Chromosome
High LDL-C	LDLR, APOB, PCSK9, STAP1, APOE, LDLRAP1, LIPA, ABCG5, ABCG8	19, 2, 1, 4, 19, 1, 10, 2, 2
Low LDL-C	MTTP, APOB, PCSK9, SAR1B, ANGPTL3	4, 2, 1, 5, 1
High HDL-C	CETP, LIPC, SCARB1	16, 15, 12
Low HDL-C	ABCA1, LCAT, LPL	9, 11, 16
High TG	APOC2, APOE	19
Low TG	APOC3	11

targeted NGS to diagnose dyslipidemias—in one instance they were able to make a genetic diagnosis in 35.9% of patients with low HDL and identified 21 novel variants related to HDL levels.[15] The results of these studies are significant as they are some of the first to validate targeted lipid NGS panels with the correct subset of genes to establish diagnoses of dyslipidemias. Another advantage of identifying genetic causes of dyslipidemias in the clinic is the opportunity to conduct genetic screening in family members. This is beneficial as it may allow for early diagnosis and management of patients whose disease may otherwise not have been apparent—it has also been demonstrated to have significant cost savings in UK FH patient populations.[16]

It is clear that we now possess a comprehensive understanding of the genetic basis of dyslipidemias to make meaningful clinical decisions based on genetic data and are approaching the technical capabilities to offer this testing in the clinic. It is important that we approach the incorporation of genetic information into routine clinical practice with realistic expectations, as challenges to diagnosing dyslipidemias still exist. The diagnosis of polygenic dyslipidemias, disorders due to multiple genetic variants, remains complex as individuals often have many common variants that each individually contribute very little to overall lipoprotein levels and thus make it difficult to identify causal variants.[17] Another caveat to consider is that although NGS may identify variants, providers may not know whether a variant is of clinical significance. Despite these challenges, one should remain optimistic about the implementation of NGS into clinical care in part due to the concerted efforts of regulatory bodies in facilitating the adoption of these technologies. In recent years, there has been the development of various standards for identifying causal variants, such as guidelines published by the American College of Medical Genetics, to help clinicians utilize genetic information.[18] In conclusion, advancements in our understanding of the genetic architecture of dyslipidemias suggest that incorporation of genetic information into clinical care would greatly improve the diagnosis and management of these conditions. NGS technologies provide the technical capabilities for clinicians to quickly obtain sufficient amounts of genetic information at a reasonable cost to inform care. This approach has been validated in numerous studies demonstrating the ability to both diagnose dyslipidemias and identify new causal variants. For these reasons, the incorporation of genetic information about lipid metabolism into routine practice ought to be seen as essential for providing quality care for patients in the future.

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