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LIPIDS AND CARDIOVASCULAR DISEASE

The role of genetic testing in dyslipidaemia

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Summary

Dyslipidaemias encompass about two dozen relatively rare monogenic disorders and syndromes for which the genetic basis has largely been defined. In addition, the complex polygenic basis of disturbed lipids and lipoproteins has been characterised in many patients, and has been shown to result from accumulation of many common polymorphisms with small effects on lipids. Genetic technologies, including dedicated genotyping and sequencing methods can detect both rare and common DNA variants underlying dyslipidaemias. Some dyslipidaemias may be clinically silent for years, but early diagnosis, including genetic diagnosis, may permit early intervention to prevent or delay deleterious downstream clinical consequences, such as premature vascular disease or acute pancreatitis. The potential clinical utility of genetic testing for familial hypercholesterolaemia, familial chylomicronaemia syndrome, lysosomal acid lipase deficiency and some others will increase demand for reliable genetic diagnostic methods. We review some current technologies, such as targeted next-generation sequencing that seem to be helpful with DNA diagnosis of dyslipidaemias. We also address technical, biological and clinical limitations of genetic testing in dyslipidaemias. Finally, genetic counselling issues, the potential impact of results on patients and health care providers, current gaps and future directions will be discussed.

Key words: Genetic testing; dyslipidaemia; familial hypercholesterolaemia; familial chylomicronaemia.

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INTRODUCTION

Increased appreciation of genetics by clinicians, researchers and the general public parallels the recognition of its causative role in many diseases. Genetic understanding is well-established for dyslipidaemias, a collection of ~25 named metabolic disorders that are characterised by extreme serum levels of lipoprotein particles such as high-, low- and intermediate-density lipoproteins (HDL, LDL and IDL, respectively) and triglycerides (TG). Inherited differences underlie both variation in lipid phenotypes in the general population and susceptibility to dyslipidaemias in patients. Furthermore, the often complex and multifactorial nature of these clinically relevant traits is becoming more evident.

The archetypal condition in which genetic analysis is often being applied clinically is familial hypercholesterolaemia (FH). FH is a common inherited disorder, with population prevalence of ~1 in 250.[1] FH predisposes to premature development of plaques in the coronary, central and peripheral vasculature that can lead to early-onset atherosclerotic cardiovascular disease (ASCVD), including coronary heart disease, stroke and limb ischaemia.[1]

Another familial dyslipidaemia is severe hypertriglyceridaemia, which can lead to acute pancreatitis that can be life-threatening.[2] Genetic analysis may be helpful in certain cases of severe hypertriglyceridaemia, such as familial chylomicronaemia syndrome (FCS). Molecular defects in other pathways involved in lipoprotein metabolism can have similarly clinically relevant effects for which genetic analysis can provide clinically actionable data.

Rapidly declining costs make it possible to consider genetic testing in selected dyslipidaemic patients or their asymptomatic relatives. However, precise indications for genetic testing for most dyslipidaemias have not yet been clearly established and there are currently no clinical guidelines for genetic testing in dyslipidaemia. We explore some issues surrounding genetic testing in dyslipidaemia, endeavouring to provide some guidance to practitioners on its role and utility in the clinical setting.

GENETIC TESTING

'Genetic testing' refers to several methods used to determine the genotype of an individual. A key factor in selecting the type of testing and technology is whether the patient is suspected to have a rare mutation of large clinical effect causing a monogenic dyslipidaemia[2] versus a collection of common single nucleotide polymorphisms (SNPs) with individually small effects that collectively create susceptibility to dyslipidaemia under a polygenic model of inheritance.[3] There are several different methods in current clinical use[4] (Table 1).

Sanger sequencing

Automated Sanger or dideoxy chain termination sequencing is used to examine small DNA segments, usually a single gene or even a single exon. It can precisely detect a single nucleotide change, often a heterozygous rare variant with a large clinical effect, in a known gene causing a monogenic disorder (we use the terms 'mutation' and 'large effect rare variant' interchangeably). This method can be applied in family cascade screening to follow the inheritance of a

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Table 1 Genetic testing methods

Method	Description	Strengths	Weaknesses	Types of detectable variation				Best use
				CNV	Rare variants	Common variants	PRS	
Sanger sequencing	Amplification using individual primer pairs in separate amplification and sequencing reactions	- Gold standard for diagnosis of small DNA changes - Highest depth of coverage	- Labour intensive - Highest cost when done in high volumes	No	Yes	No	No	- Single gene tests - Confirmation of presence/ absence of a known mutation in familial cascade testing
Targeted NG sequencing	Simultaneous screen of a pre-selected subset of genes	- Maximise diagnostic yield and minimise off-target results - High depth of coverage	- Will not find mutations in genes outside the panel design	Yes[a]	Yes	Yes[b]	Yes[b]	- Investigating a condition with multiple causal/contributing genes
Whole exome sequencing	Simultaneous screen of all exons (i.e., coding sequences) in an individual (2% of total DNA)	- Can identify new or unexpected genes - Could be re-examined for other conditions at a later date	- Potential for off-target results - Higher probability of uncertain results	Yes[a]	Yes	No	No	- Conditions with an unclear genetic basis - Conditions expected to be genetic but without a cause identified on other testing
Whole genome sequencing	Simultaneous screen of all coding and noncoding DNA in an individual, including mitochondrial	- Includes all genetic material, including regulatory regions - Could be re-examined for other conditions at a later date	- Lower depth of coverage - Higher probably of uncertain and off target results - Labour intensive	Yes	Yes	Yes	Yes	- Same as for whole exome but provides more comprehensive data
Genotyping	Rapid screen of entire genome using an array of common variant SNPs	- Rapid - Lower cost - Entire genome examined	- Cannot detect novel variants - No sequence-level data	Yes	Yes[b]	Yes[b]	Yes	- Can be used to look for disease risk, ethnicity and to determine familial relationships
Polygenic risk score	Derived by summing the minor effects of several common variants to generate an overall estimate of risk	- Can provide information on risk that will not be apparent with other forms of sequencing	- Ethnic variations can limit use - Predictive value varies with each score	N/A	N/A	N/A	N/A	- Used as a complement to traditional sequencing to help provide an additional clinical prediction of risk

CNV, copy number variant; PRS, polygenic risk score.
[a] With specialised bioinformatics tools.
[b] If designed to target.

previously identified mutation. Sanger sequencing has the added benefit of providing only the desired genetic result with little to no chance of incidental findings. The cost of Sanger sequencing varies depending on the size and number of genes and exons tested, with each 1–2 kilobase length of DNA requiring: (1) design and purchase of a primer pair (~$30 USD); and (2) polymerase chain reaction (PCR)-based bi-directional sequencing (~$20 USD). Both in the clinic and research lab, Sanger sequencing often serves as the 'gold standard' confirmation when a potentially causative variant is found with other methods.[5,6]

Next-generation sequencing

Next-generation sequencing (NGS) refers to various methods that each use a massively parallel sequencing design to amplify and examine multiple segments of DNA concurrently.[7] This technique is typically applied to detect a rare causative variant for a monogenic condition when many possible mutations exist, and can be used to sequence: (1) a targeted selection of pre-specified genes; (2) all expressed protein-coding sequences ('whole exome sequencing' or WES) representing ~2% of the entire genome; or (3) all coding and non-coding regions comprising the entire genome ('whole genome sequencing' or WGS).[7,8] WES is optimal for detecting rare coding variants primarily, while WGS detects all common and rare variants in all coding and non-coding regions, producing an enormous data file for each sample.

Targeted NGS panels can be designed to both screen for rare variants in coding regions and concurrently evaluate non-coding common SNPs as part of a polygenic risk score. Several targeted panels have been clinically validated and their cost is decreasing steadily.[9] The cost of this technology varies depending on how many genes are concurrently assessed, with approximate costs per sample of $300 for a targeted panel, $800–1200 for WES and $3–10,000 for WGS.[8] For genetic analysis of dyslipidaemias, targeted sequencing panels are the current standard for clinical diagnosis.[2,10–12]

Genotyping

Genotyping refers to various types of dedicated, inexpensive methods to directly assay specific known rare variants or common SNPs. Many different technical and chemical platforms can be used for dedicated genotyping. Depending on the indication, these methods can evaluate a single or numerous pre-defined SNPs already known to be associated with certain dyslipidaemias. A familiar example would be genotyping of *APOE* isoforms to identify E2/E2 homozygosity in suspected type 3 hyperlipoproteinaemia (dysbetalipoproteinaemia).

Large-scale extensive genotyping employs high density genome-wide microarrays in unbiased genome-wide association studies (GWAS) to discover associations between millions of SNP markers and either quantitative or qualitative complex clinical traits.[7] Once a subset of SNP genotypes has been definitely associated with clinical phenotypes, a smaller, less costly panel can be constructed using dedicated chemistry (e.g., TaqMan), mass spectrometry or custom-made microarrays, allowing focused testing of clinical samples. Genotyping methods detect only those DNA changes they have been designed to capture;[7] however, for clinical applications, there is no need to screen for every possible base pair variation or to detect a rare unknown mutation. Many commercial direct-to-consumer DNA tests employ SNP genotyping.

Polygenic risk scores using SNPs

Dyslipidaemia sometimes results from accumulation of multiple small-effect DNA variations. These common allelic variants each only slightly affect lipid levels, but when present in sufficient numbers in an individual's genome they can underlie a phenotype resembling that of an individual carrying a single, rare, large-effect mutation.[13–15] For example, several SNPs scattered throughout the genome have been shown in GWAS to have modest but highly reproducible effects on lipid traits, e.g., HDL cholesterol (C), LDL-C or TG.[3] Most people have some SNP alleles that raise and others that lower a lipoprotein trait, resulting in an average serum level. However, some individuals, by unlucky chance, inherit an excess of SNP alleles that concertedly alter the lipid trait in the same direction (e.g., they all raise LDL-C).[16] High cholesterol from accumulation of many small-effect SNPs can be indistinguishable clinically from a single gene rare variant cause.[17] However, this mechanism of disease is not detected by some forms of genetic testing, e.g., WES or targeted sequencing that is not designed to concurrently detect common associated non-coding SNPs.[16,17] These lipid trait-altering SNP genotypes can be assessed using dedicated allele-specific detection methods, such as TaqMan-based assays or SNP microarrays, or by designing targeted NGS panels to detect them.

Once these SNPs have been genotyped, they are entered into a scoring scheme to quantify their cumulative effect on the lipid trait. These scores sum the expected effect of each individual SNP in a given patient, yielding a polygenic SNP score (also called a 'polygenic risk score' or 'polygenic trait score') that can be compared to a normolipidaemic population. An individual with a high polygenic score, e.g., >90th percentile for the population, would be predisposed to develop clinically apparent dyslipidaemia.[16,17]

Since targeted NGS is the method of choice to detect rare mutations, a portion of excess capacity on the panel could be designed to concurrently sequence non-coding regions harbouring key SNPs needed for a polygenic risk score. This means that both rare and common DNA variation underlying clinical dyslipidaemia can be concurrently evaluated using a single laboratory method, which saves time, effort and expense.[2] Using NGS to generate polygenic risk scores adds information on the underlying genetic contribution to a phenotype.[2] We have shown that it fills in the missing genetic gap for many disorders, such as in FH, where up to one-third of clinically-ascertained cases without a discrete rare large-effect mutation actually have a high polygenic score;[17] similar findings are seen in patients with extreme levels of HDL-C.[18]

SNP scores have been generated using a small number of SNPs, several hundred SNPs and recently several million SNPs;[15] the score's predictive value has been demonstrated to increase with the number of SNPs incorporated. There is no current consensus for how to use polygenic risk scores in dyslipidemia diagnosis. Since many dyslipidaemic patients have a polygenic rather than monogenic aetiology, polygenic

risk scores could play an increasing role in genetic diagnosis,[15] especially if they can be shown to predict clinical outcomes[15] or response to interventions.

Copy number variation

The term copy number variation (CNV) refers to a specific quantitative type of genetic variant characterised by altered dosage of a genomic region, i.e., deviation from the usual two copies of a particular genomic sequence. CNVs can be large-scale duplications or deletions that affect a whole exon, gene or even several genes. They are usually sub-chromosomal in size and can cumulatively affect up to 10% of the genome, compared to only 1–2% of total genomic DNA that varies due to all known SNPs and small-scale rare variants.

CNVs in the *LDLR* gene account for up to 10% of causal FH mutations, making it important to check for these in the diagnosis of FH.[19] CNVs are difficult to directly detect with DNA sequencing, especially large deletions, which eliminate one copy of normal sequence that cannot be amplified and chemically processed by Sanger methodology. In this instance, only the single normal allele is read, but there is no way to tell that the second allele is missing. Similarly, an extra copy of a long stretch of genomic DNA is difficult to detect by sequencing methods that are optimised to identify small-scale variation. In the past, separate methodology, such as high definition cytogenetic analysis, or comparative genomic hybridisation with DNA microarrays or multiplex ligation-dependent probe amplification (MLPA) was required. For diagnostic laboratories, establishing an additional method to detect CNVs increased costs: e.g., MLPA of the *LDLR* gene costs ~$100 USD per sample on top of the sequencing cost.

Fortunately, new bioinformatic tools can detect CNVs from raw NGS data, without additional reagents.[19] Targeted NGS can thus detect rare, large-effect, small-scale variants and CNVs causing monogenic dyslipidaemias, in addition to SNPs used in polygenic risk scores. The ability to simultaneously evaluate several forms of variation using a single laboratory method improves diagnostic yield (i.e., fewer false negatives), without increasing the cost.

Other advanced genetic tests

Other methods may provide additional useful clinical information for dyslipidaemias. These techniques include: (1) proteomic methods, whereby protein expression is assessed at the cell or tissue level to indirectly evaluate gene expression; (2) metabolomics, which refers to the concurrent assessment of small molecule metabolites from serum or tissue samples to indirectly evaluate in a targeted manner variations in gene products involved in intermediate biochemical pathways or in drug metabolism;[20,21] and (3) genome-wide transcriptome analysis of RNA expression (e.g., RNASeq) that can be further used to assess gene expression and patterns of alternative RNA splicing within a cell or tissue.[22]

Epigenetics is increasingly recognised as affecting genotype-phenotype relationships. Incorporating epigenetic analysis using methylation arrays[23] or other assays into standard genetic practice may be on the horizon.[9] However, for dyslipidaemias this is limited by the fact that epigenetic imprinting in relevant tissues such as liver or intestine is not faithfully reflected by patterns in white blood cells, which are the usual convenient source of genetic material.

Determining pathogenicity

Even when a rare variant in a known causative gene for monogenic dyslipidaemia is detected, its causality or pathogenicity is not guaranteed. DNA changes may be synonymous, meaning that they code for the same amino acid as the base pair that was replaced, or if non-synonymous, they may still be benign, in that the altered amino acid has no clinically relevant impact on the structure or function of the translated protein.

The highest grade of evidence for a mutation's pathogenicity is a functional study performed in a research laboratory showing that the mutation causes a measurable change in an assay or model system.[24] Familial segregation analysis that shows complete genotype-phenotype concordance is also helpful; i.e., each affected family member carries the variant, while unaffected members do not carry the variant. The evidence becomes statistically stronger as more family members are tested. Conversely, the presence of the mutation in healthy, phenotype-free individuals argues against pathogenicity.[24]

A second tier of evidence for pathogenicity comes from 'bioinformatics', i.e., computational modelling and analysis. Here, prediction software tools employ complex but validated mathematical algorithms to predict the impact of the amino acid change based on the degree of evolutionary conservation of the wild-type amino acid, the predicted 3-D structure of the resultant protein, and other circumstantial information.[24] However, these methods sometimes give inconsistent results for the same mutation. Even after manual adjudication by human genetics experts, there can be disagreement regarding a mutation's potential pathogenicity. Nonetheless, trained, knowledgeable and skilled individuals must be available as back-up to interpret results of genetic tests, both to avoid incorrectly assigning pathogenicity to a benign change or incorrectly ascribing neutral function to a truly pathogenic variant.

Potential benefits

Genetic testing, in the best case, can provide a definitive diagnosis. Possible benefits include tailoring management to the individual patient (Table 2). For instance, heterozygous FH from CNVs seems to be a more severe phenotype than FH from single nucleotide variants.[17,25] Genetic diagnosis could reduce delay in selecting an appropriate treatment, i.e., possibly more aggressive LDL-C lowering strategies.[21] Additionally, for some monogenic dyslipidaemias, a genetic diagnosis is needed to secure funding for newer therapies. For instance, in certain jurisdictions, third party private coverage for injectable inhibitors of proprotein convertase subtilisin kexin 9 (PCSK9) for the treatment of FH requires a genetic diagnosis.

Some genetic dyslipidaemias also carry risk for other outcomes, such as hepatic cirrhosis in cholesteryl ester storage disease (CESD) or Wolman disease due to rare bi-allelic *LIPA* variants,[26] where third party coverage to receive sebelipase (intravenous lysosomal acid lipase infusion) often depends on a positive DNA diagnosis. Other genetic conditions can mimic a classic dyslipidaemia but because they are

Table 2 Clinically actionable impact of genetic diagnosis in selected monogenic dyslipidaemias

Condition	Causative gene(s)	Management effect
Familial hypercholesterolaemia	*LDLR, APOB, PCSK9*	Insurance eligibility for monoclonal antibody inhibitors of proprotein convertase subtilisin kexin 9 (PCSK9), e.g., evolocumab (Repatha) or alirocumab (Praluent) or lomitapide (Juxtapid - inhibitor of microsomal triglyceride transfer protein) Treatment selection (e.g., mutant receptors that retain partial function may respond to certain treatments in homozygous FH)
Lipoprotein lipase deficiency	*LPL*	Alipogene tiparvovec (Glybera - *LPL* gene therapy)
Familial chylomicronaemia	*LPL, APOC2, APOA5, LMF1, GPIHBP1*	Volanesorsen (Waylivra - antisense inhibitor of apolipoprotein C-III)[a] Lomitapide (Juxtapid - small molecule inhibitor of microsomal triglyceride transfer protein)
Wolman syndrome (cholesterol ester storage disease)	*LIPA*	Sebelipase alfa therapy (Kanuma - intravenous enzyme replacement for lysosomal acid lipase)
Analphalipoproteinaemia	*APOA1*	Recombinant human apolipoprotein A-I[a]
Familial lecithin cholesterol acyl transferase deficiency	*LCAT*	Recombinant human lecithin cholesterol acyltransferase[a]
Cerebrotendinous xanthomatosis	*CYP27A1*	Oral cholic acid and chenodeoxycholic acid

[a] Not yet available on the market.

treated differently, a genetic diagnosis can be helpful. For instance, sitosterolaemia sometimes resembles FH, but its management focuses on intestinal cholesterol absorption inhibition rather than statins.[27]

Furthermore, patients with some dyslipidaemias do not express overt symptoms, such as atherosclerosis, until later in the disease course. When early prevention and management have been shown to delay onset of complications, there is benefit to identify and treat these individuals assertively as early as possible, as with heterozygous FH.[28]

Furthermore, opportunities may arise for early detection and intervention for children or family members of individuals with a discrete monogenic dyslipidaemia. For instance, heterozygous FH follows an autosomal dominant transmission pattern, meaning that half of all first-degree relatives also have the condition.[29] Many FH individuals in the pre-clinical stage of ASCVD would benefit from an early diagnosis. A definite DNA diagnosis could also impact on family planning, especially for the parents of children presenting with severe homozygous dyslipidaemias; a similar presentation would be expected in one-quarter of future offspring. There may also be benefits for adults who are considering starting a family and wish to evaluate potential risks, which can be explained by a genetic counsellor.

Potential non-clinical benefits of genetic testing include patient empowerment. Knowing their diagnosis, even without interventions or prevention strategies, can still provide awareness of the expected disease course, allowing for advanced planning, and emotional and mental control over healthcare.[30] A negative test result for an at-risk individual may provide psychological relief and reduces costs of future surveillance.[30]

Systematic genetic testing in an academic clinic or a disease registry can also improve understanding of the pathophysiology and consequences of a condition. This in turn can lead to the development of new, more effective pharmacological treatments or management strategies. A prime example is the development of PCSK9 inhibitors, which owe their inception and development to genetic studies of altered lipids in patients, families and communities.

Potential drawbacks

A major limitation to genetic testing is potentially prohibitive costs, especially for widespread WES or WGS. However, costs are dropping rapidly, as predicted by Moore's law of the economics of new technologies.[9] The first completed whole DNA sequence in 2000 cost $2.7 billion; the cost today is $3–10,000 or less for a whole genome and much less for a whole exome. As costs continue to decline, this barrier is increasingly overcome.

Ethical dilemmas can arise from increased use of genetic testing. Researchers are familiar with unexpected off-target results in WES or WGS, i.e., incidental findings unrelated to the disease of interest. As these findings arise more commonly in the clinic, a policy is required regarding communication to patients. For example, if genetic testing in a patient with dyslipidaemia were to incidentally pick up a mutation in the familial breast cancer *BRCA1* gene, what is the obligation to report it? Furthermore, hundreds to thousands of incidental variants of uncertain significance (VUSs) in many genes are picked up with both WES and WGS.[24] What is the most appropriate way to evaluate and communicate these findings? A conflict of interest might arise between the researcher's or physician's 'duty to inform' at-risk patients and family members, and the individual's 'right not to know'.[31,32] Furthermore, genetic testing could infringe on family privacy, since results indirectly provide information about family members.[31] The American College of Medical Genetics and Genomics recommends that incidental findings for certain medical conditions should be communicated to patients.[33]

Genetic information might also affect eligibility for work or insurance, or could otherwise lead to 'genetic discrimination'.[31,34] Legislation in many countries, including the United States and Canada, is intended to protect against genetic discrimination in the workplace, and by insurance providers. However, there is little consensus regarding who can access genetic information and for what purpose.

A commonly held misbelief is that genetic testing offers diagnostic certainty when either a positive or negative result is reported. However, genetic testing is just as prone to false positives and negatives as other diagnostic tests, although

precise sensitivity and specificity of genetic testing often cannot be determined (e.g., due to lack of reference standards).[34–36] Overestimation of the 'power' of genetics can lead to both unnecessary anxiety in the case of a false positive (e.g., when a benign VUS is labelled as pathogenic, or when two causal mutations for a homozygous trait reside on the same allele but are interpreted as compound heterozygote and thus disease-causing), or inappropriate reassurance with a false negative result (e.g., for a mutation in a gene not yet associated with the disease).[36]

There are also financial issues to consider. While the number of clinically validated tests available is increasing, the guidelines and indications for their use and interpretation is slower to emerge, as are standards for reimbursement by insurance companies and governments.[9,30]

When to test

The case for genetic testing is strengthened when there is strong suspicion (i.e., no obvious secondary causes, a strong family history, values far outside standard reference ranges, other suggestive syndromic features, or a young patient) AND when there might be a change in management, monitoring or intervention that could affect outcomes for the patient or family members (i.e., will effect eligibility for a new drugs, would potentially lead to a different choice of therapy) OR if there is a strong patient desire for a definitive diagnosis (Table 3).[30,36,37]

FH is the best documented example of monogenic dyslipidaemia.[35] It is caused predominantly by >2000 reported mutations in the *LDLR* gene encoding the LDL receptor. Pathogenic mutations are found in all 18 exons and the promoter region.[1,29,35,38–40] Mutations in other genes, including receptor binding defective mutations in *APOB* encoding apolipoprotein (apo) B and gain-of-function mutations in *PCSK9*, are minor contributors.[1,29,35,38,39] Rare autosomal recessive mutations in *LDLRAP1* encoding LDL receptor adaptor protein 1 can also underlie an FH phenotype.[38]

Criteria for testing are well-established and are sometimes suggested for anyone with an LDL-C level >5.0 mmol/L (>90th percentile) and having at least one of the following: (1) a family member with known FH or elevated cholesterol; (2) physical findings of FH including tendon or plantar xanthomas, xanthelasmas, and corneal arcus in an individual <45 years or their presence in a family member; (3) premature coronary heart disease (onset <55 and <65 years in men and women, respectively) in the individual or family member, or a family member with sudden premature cardiac death; or (4) if the patient meets criteria for possible or probable FH by the Simon Broome or Dutch Lipid Clinic Network criteria.[21,29,38,39,41] If an individual is identified as FH mutation-positive, screening of first-degree relatives is advised.[1,41]

The benefits of establishing a genetic diagnosis of FH include the ability to intervene early to minimise the future cardiovascular disease risk. Patients with genetic FH are at higher risk of premature ASCVD events than individuals with similar LDL-C levels but no rare variant driver for their hypercholesterolaemia.[42,43] Medications, such as statins, ezetimibe, evolocumab and alirocumab, improve and sometimes even normalise LDL-C levels in FH individuals. Observational studies suggest that early intervention in this population significantly reduces the lifetime risk of ASCVD.[28,44,45] Genetic testing is recommended by several guidelines as standard for FH diagnosis; it is often more accurate and definitive than phenotypic assessment alone.[41] Some guidelines suggest universal FH screening; however, this has practical and economic limits. Biochemical rather than genetic cascade screening of family members of a known FH mutation carrier can be sufficient to screen for additional affected individuals.[38,41,44,46]

There are currently no guidelines for genetic testing in other monogenic dyslipidaemias. If available, we suggest testing should be based on clinical suspicion from abnormal laboratory findings, physical findings or syndromic features, or suggestive family history. An overview of the features of genetic dyslipidaemias is reported in the article by Ng *et al.* in this issue.[47]

Table 3 Possible indications for genetic testing in dyslipidaemias

Testing might change management
Strong clinical suspicion
Patient preference
Family planning
Early interventions available
Eligibility for new drugs
Strong family history
Other, related syndromic features

GENERAL GUIDELINES FOR GENETIC TESTING: PITFALLS

As mentioned, an apparently negative genetic test does not rule out an underlying genetic cause. For instance, a 'silent' appearing variant may actually affect splicing or epigenetic modification. There may be undetected pathogenic changes (e.g., deep within introns) that are not yet linked to the disorder. Without a gold standard reference test, the false negative rate of genetic testing is difficult to quantify and varies between conditions.[4] For example, mutations are found in 60–90% of individuals classified clinically as having 'definite FH', but only in 20–30% of patients classified as 'probable FH'.[38,48]

False positive results also occur.[49] Many detected rare mutations are not disease-causing, even when they alter the coding sequence of a known disease-related gene. Efforts to standardise diagnostic criteria for FH mutations are in progress: an FH mutation working group has created guidelines for *LDLR* mutation pathogenicity.[10,39] This may help standardise FH diagnosis worldwide, and may be useful to replicate in other disorders. The inter-assessor concordance rate using these criteria was high, but was not 100%.[10]

Also, with WES and WGS there is risk of incidentally detecting mutations in off-target genes unrelated to the referring diagnosis.[50] Furthermore, reporting risk or susceptibility mutations without available interventions could cause unnecessary anxiety and expend resources, without any compensatory benefits. Along these lines, a recent study on WGS of apparently healthy Canadians found that ~25% of them had 'clinically actionable' mutations,[51] although there was no definitive benefit in knowing this information; more work is required.

As mentioned, discovery of VUSs by high-throughput methods is common.[52] These are often previously

unreported mutations detected in an individual presenting with a disease that were not previously known to be pathogenic. Imputing causality bioinformatically is usually not helpful, and much work is needed to improve the clinical approach to such findings. Ultimately, these findings will likely have limited clinical utility.[52]

IMPACT ON MANAGEMENT

Do genetic test results affect clinical management? Some studies report that providing genetic test results to clinicians did not affect practice.[37,53,54] This may also hold true for FH genetic testing; specific *LDLR* mutations do not correlate consistently with disease severity or ASCVD risk.[10,29,35] In a study comparing lipid clinic health care providers who either had or did not have access to genetic testing results,[53] clinicians reported the general impression that current genetic methods were imperfect for FH diagnosis. They believed that 10–50% of FH patients who formally tested as 'mutation-negative' still had an unidentified FH mutation.[53] Clinical diagnosis of FH was consistently judged as more important than DNA testing: a negative genetic test result did not rule out FH when clinical suspicion was strong.[53] Many felt lipid levels alone were sufficient to guide intervention; genetic test results did not change management.[53] The main impact of genetic test results would be on the family members of affected individuals, especially children who could be pre-symptomatically diagnosed.[53] These findings might have been different had the study been conducted in the PCSK9 inhibitor era.

IMPACT ON PATIENTS

The impact of genetic testing on patients depends on several factors, including disease-specific factors, past personal experiences, the method of information delivery, and patient-specific factors.[46] Some studies report a lack of retention or understanding with respect to genetic risk. For example, a study of Alzheimer's susceptibility testing found that only 27% of patients tested could accurately recall their results a year later and 23% were unable to recall any information conveyed at the time of testing.[55] Qualitative studies suggest a wide range of responses to genetic information depending on the strength of the genetic findings, penetrance of the disease, whether the individual has witnessed affected family members, or whether active disease symptoms are present.[53,56]

In a study of newborn FH screening, elevated cholesterol levels in FH that was diagnosed based on biochemical criteria were interpreted as being more controllable and less distressing than if FH was diagnosed using DNA testing.[57] In adults, disclosure of FH status was reported as being no more or less important than other cardiovascular risk factors.[57] One study found that patients with an FH diagnosis felt less responsible for their dyslipidaemia; they seemed to interpret their dyslipidaemia as being distinct from high cholesterol in other individuals that was perceived to be lifestyle-related.[53,58] Promising results were seen in patients undergoing cardiovascular risk stratification randomised to receive information on either conventional risk factors alone or conventional risk factors plus their genetic risk profile. Individuals with high genetic risk were more likely to remain on their statins than those at low genetic risk or those who were not informed of their genetic profile.[20]

GENERAL GUIDELINES FOR GENETIC TESTING: HOW TO COUNSEL

Counselling regarding genetic testing encompasses two domains: medical and psychological.[24] Medical counselling focusses on patient education regarding the expected health impact of the mutation, explanation of disease course and management, addressing modifiable risk, and reproductive implications.[59] Psychological and emotional support are particularly important in untreatable diseases, or if there are severe health manifestations; these involve making decisions that are more personal than medical.[59] Who should provide counselling surrounding a genetic test result? Should this be up to the ordering physician, a specialised genetic counsellor, the family physician or another provider? Does the reporting laboratory play a role in counselling or education? For dyslipidaemias, genetic testing is often obtained through specialised lipid clinics, so these may be the best sites to provide counselling.

PRACTICAL STEPS IN CLINICAL GENETIC TESTING

Counselling should be available throughout all stages of genetic testing: pre-testing, during sample collection, post-testing and in follow-up. Pre-test counselling should focus on the informed consent process, discussing the testing procedure itself as well as the objective of testing and the risks, benefits, limitations and consequences of testing.[24,31,52] The reliability and interpretation of the test should be discussed. Possible implications of a positive test result on the patient and family members should also be reviewed, including potential social and emotional effects.[31] False negative and positive detection rates should be acknowledged, as well as the potential for incidental findings and VUS. Possible psychological ramifications, such as survivor or parental guilt, should be discussed.[24] Attempt should be made to evaluate baseline anxiety levels, perception of risk, and health attitudes and beliefs.[24]

During sample collection, the patient's willingness to proceed should be confirmed, the test procedure reviewed and any questions answered before obtaining the sample. Post-test counselling includes confirming that the patient still desires their result prior to disclosure. The specific findings and their impact on the patient personally as well as his/her family members should be a major focus.[52] Clinical screening and management recommendations should be addressed.[24] Opportunity for questions and reflection should be provided. A discussion on cascade testing should be provided if a mutation is detected. Emotional and psychological support should be provided as required. Assistance in locating and disclosing genetic test results to family members could be offered.[24,32] At follow-up, many of the same principles apply. Ongoing support and information should be provided as required.

CONCLUSIONS

The potential benefits of genetic testing include the increased likelihood of a definitive diagnosis for a patient or family, opportunity to improve management and disease prevention strategies and to help advance overall knowledge and understanding of complex metabolic pathways and processes. The decision to proceed with genetic testing in a particular

patient should focus on a global assessment of the individual that takes into account baseline suspicion of a disorder coupled with the expected benefits of the test results, and may depend strongly on individual patient values and preferences. An understanding of the limitations of current genetic testing techniques is important when deciding to proceed with testing and for proper interpretation of the results. Ultimately, genetic testing can provide a powerful and informative tool to aid in clinical diagnosis and management, as well as in advancing care strategies beyond currently available treatments, but should be used in appropriately selected patients, by clinicians who can accurately interpret the results, to achieve the greatest benefit.

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