

REVIEW

Genetic Testing for Dyslipidemia in Children – Ethical Challenges and Dilemmas

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ABSTRACT

The expanding appreciation of genetics by researchers, by clinicians and the general population goes hand in hand with the acknowledgement of its role in a multitude of diseases. However, each newly developed genetic test raises conflictual concerns in medicine, public health and social policy regarding the medical approaches under which the test would have a valuable role, and what are the uses of its results.

Genetic understanding is well defined for dyslipidemias, a collection of metabolic disorders which is characterized by high levels in the blood of lipoproteins, HDL, LDL and triglycerides (TG). The transmitted differences among families underlie the variation of lipid phenotypes and susceptibility to dyslipidemia. Disorders of lipid metabolism caused by genetic mutations along with other acquired risk factors are common in children. The nature of clinical features is often multifactorial and complex. While some show clinical signs and symptoms, children with genetic mutations, such as familial hypercholesterolemia (FH), are asymptomatic and generally normal weight, but increased cholesterol throughout life plays a key role in the progression of atherosclerosis from childhood and increases the risk of developing cardiovascular diseases such as myocardial infarction and stroke.

When considering FH, one of the most common monogenic diseases, there are unique benefits in identifying the causal genetic variant of patients under the age of 18th, because the presence of elevated levels of persistent cholesterol formed on an early age leads to the formation of atherosclerosis and participates in its progression towards adulthood. When identifying a child at risk, disease prevention is done through proper assessment, early formation of a healthy lifestyle, and appropriate medication to lower lipids. The risk can be greatly reduced in order to prevent future events related to atherosclerotic cardiovascular disease¹.

Keywords: familial hypercholesterolemia, children, ethics, genetics.

REZUMAT

Aprecierea din ce în ce mai crescută a geneticii de către clinicieni, cercetători și a populația generală merge mână în mână cu recunoașterea rolului său în multe boli. Cu toate acestea, fiecare test genetic nou ridică preocupări conflictuale în medicină, sănătate publică și politică socială cu privire la abordările medicale în baza cărora testul ar avea un rol valoros și care sunt utilizările rezultatelor sale.

Înțelegerea genetică este bine stabilită pentru dislipidemii, o colecție de tulburări metabolice care se caracterizează prin niveluri serice ridicate de lipoproteine (HDL, LDL) și trigliceride (TG). Diferențele transmise stau la baza variației fenotipurilor lipidice și a susceptibilității pentru dislipidemii, natura trăsăturilor clinice este adesea multifactorială și complexă. În timp ce unii prezintă semne și simptome clinice, copiii cu mutații genetice, cum ar fi hipercolesterolemia familială (HF), sunt asimptomatici și, în general, sunt cu greutate normală, dar creșterea colesterolului de-a lungul vieții joacă un rol cheie în progresia aterosclerozei din copilărie și crește riscul de a dezvolta boli cardiovasculare, cum ar fi infarctul miocardic și accidentul vascular cerebral.

Luând în considerare HF, una dintre cele mai frecvente boli monogenice, există beneficii unice în identificarea variantei genetice cauzale a pacienților cu vârsta sub 18 ani, deoarece prezența unor niveluri crescute de colesterol persistent format la o vârstă fragedă duce la formarea aterosclerozei și participă la progresia bolii către maturitate. Atunci când se identifică un copil cu risc, prevenirea bolilor se face printr-o evaluare adecvată, formarea timpurie a unui stil de viață sănătos și tratament adecvat pentru scăderea lipidelor. Riscul poate fi mult redus pentru a preveni evenimentele viitoare legate de boala cardiovasculară aterosclerotică.

Cuvinte cheie: hipercolesterolemie familială, copii, etică, genetică

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INTRODUCTION

What rights does the child have in deciding whether to undergo testing? The purpose of the article is to discuss genetic testing from the perspective of minor patients' rights, as an ethical and legal necessity.

We present concepts of genetic testing, genetic counseling and effective interpretation of results, addressing the specifications young people and evaluating future directions of pediatric lipidology in the field of diagnostic genetics.

The decision to perform genetic testing requires a better understanding of the details associated with the young population. They have the potential to provide valuable information for assessing clinical management, especially in disease prevention and patient education for selfcare as future adult with a high-quality life. Although still underused, genetic testing identifies causal variants in disorders of lipoprotein metabolism and provides confirmation of disease diagnosis, risk classification, possible identification of high-risk family members, and individualization of treatment options.

The rapid decrease in costs makes it possible to test dyslipidemia patients and asymptomatic relatives. However, there are still no clinical protocols for genetic testing in dyslipidemia, and several variants are used to determine a person's genotype. The important factor in selecting the type of test is whether the patient is suspected of having a rare, monogenic mutation with a high clinical effect that causes dyslipidemia, compared to a collection of common single nucleotide polymorphisms (SNPs) with individual effect, which creates in cumulatively susceptibility to dyslipidemia with polygenic inheritance model.

GENETIC TESTING METHODS

Genetically inherited dyslipidemias can be classified as monogenic or polygenic. When considering conditions with known genetic loci for FH, the primary test method often screen for monogenic dyslipidemias by analyzing a panel of associated genes through sequencing and deletion/duplication analysis. At least four genes in sterol and lipoprotein pathways, LDLR, APOB, PCSK9 and LDLRAP¹, are displayed in varying gene-dose effect to cause FH. Using a combination of NGS technologies and Sanger sequencing, the targeted panels analysis is performed to identify smaller deletions or duplications and genetic variation in exons, often with sensitivity and specificity of 99%².

Sanger sequencing is the first used technology to accurately detect a rare heterozygous variant, a nu-

cleotide change in a known gene that causes a monogenic disease. It is advantageous in providing only the desired genetic result, with little chance of accidental secondary discoveries. The method can be applied in family screenings to follow the inheritance of the initially identified mutation. In both clinical phenotype and the genetic research laboratory, Sanger sequencing still serves as the „gold standard” for diagnostic confirmation when a potentially causal variant meets other test methods.

Next-generation sequencing (NGS) is the new technology which offers various methods that each use a massive sequencing project to amplify and examine several DNA segments simultaneously. This technique is used to examine rare causal variants for monogenic conditions and to sequence targeted pre-specified genes.

The main test methods are Whole Genome Sequencing (WGS) and Whole Exome Sequencing (WES). WGS detects all common and rare variants from all coded and uncoded regions that comprise the entire genome, producing a huge data file for each sample, and WES represents all coded sequences of expressed proteins (2% of the entire genome). WES detects rare coding variants and allows sequencing of regions that encode exons in the human genome, and it is often performed when the differential diagnosis is unclear, or after a negative targeted genetic test. In FH, WES can be useful when no known variant is found in a targeted panel, as several other conditions that affect lipid metabolism with known genetic variants can produce an FH phenotype, in which associated with variants of these genes create an overlap in elevated LDL levels with those observed in pathological carriers of FH variants.

It is important to note that when WES is performed, secondary findings may occur for variants in genetic sites unrelated to the suspected condition. The target panels and WES have the capacity to identify unintended secondary discoveries as side effects. For example, suspicion of FH with BRCA1 and BRCA2 variants, associated with breast or ovarian cancer predisposition in a patient and may imply the possibility of family transmission. When identifying secondary findings, patients need reference to the geneticist or relevant medical specialist. Understanding the classification of new discovered genetic variants presents an algorithm. Each genetic testing laboratory has its own data base and offers its own classification for the newfound variants, one laboratory can define a variant as benign, while another can define the same variant as

pathogenic. As technology advances, the classification for each variant continuously changes with additional close-up data on new variants³.

The interpretation of a pathogenic classification in FH is simple. The observed variant is considered to be the cause of the phenotype if the new variant type found was previously identified in individuals with the same variant. Interpretation complicates itself in cases of variants of unknown significance (VUS). In these cases, it is essential to consider how important the additional data information is in determining a causal link between an VUS and the clinical condition. Family testing provides a valuable additional data to help classify the findings in question more accurately and to guide clinical judgment for medical decision. In cases of negative results, it is important to understand that each test method chosen has its own limits. If a targeted panel for FH is ordered and no pathogenic variant is identified, the chosen test may not include all known variant sites, or there are other potential additional variants, then additional testing, like WES, can be helpful⁴.

Direct-to-consumer tests are not regulated and results should be corroborated with clinical testing. Laboratories that perform clinical genetic tests require certifications to ensure accuracy.

RESULTS AND DISCUSSIONS

A large part of the general population contains, other than LDLR, APOB, PCSK9 and LDRAPI, associated variants of genes at high risk for cholesterol and cardiovascular disease. SNPs are common variants of single nucleotides in several low-effect genes or genetic locations that do not independently affect the phenotype, but when expressed cumulatively, cholesterol or triglyceride levels may be elevated. SNPs serve as a basis for genetic risk scores and genome-wide association studies, which target the correlation of common genetic variations associated with the presence of the phenotype and define the risk of a specific genetic profile. Polygenic risk scores use algorithms that aggregate SNPs associated with certain outcomes, like increased levels of LDL or TG in the individual's profile.

By combining universal phenotypic screening established in childhood with genetic testing and parental testing, there is the potential to identify every existing case of FH within a generation of testing. And so, subsequent testing of children of affected patients would identify future cases.

Scientific evidence for and against the introduction of screening programs for genetic testing can be clas-

sified into four categories, including ethical concepts:

- clinical efficacy exemplify screening procedures that involve maximum benefit minimum risk;
- effectiveness depends on patients' cooperation to follow clinical recommendations;
- the optimal receptivity of the patients can transform an effective procedure at individual level into an efficient one at population level;
- symptomatic individuals are more cooperative than asymptomatic, and so the real test of effectiveness is given by the asymptomatic population.

These 4 abstract concepts were converted in relevant questions in order to evaluate the validity of a genetic test as a screening method: are screening procedures able to detect anomalies with a major population impact? Can the treatment of risk factors change the evolution of the disease? How cooperative are patients in administering treatment as a result of the genetic screening test? Are the methods used in the evaluation of the clinical effectiveness of screening programs likely to lead to false conclusions? Do existing screening programs result in a decrease number of cases detected after their practice over long periods of time?

Understanding the bioethics cardinal principles guides the medical decision in choosing the context when genetic tests are recommended, especially for children, as the geneticist analyzes every medical case individually:

1. **autonomy** – respecting the choice of the individual, the right to decide alone based on informed consent, with no constraints;
2. **privacy** – benefit and non-abuse of the individual;
3. **confidentiality** – preserving respect for privacy for the individual;
4. **equity** – every person's access to medical services, ensured that all individuals will be treated fairly and equally⁵.

The answers to these questions depend on the sense given to the four ethical and legal principles described and guides the process of taking medical decisions. Genetic counseling begins before performing the genetic testing when the patient and the parents are informed about the suspected condition, how genetics can benefit and the potential for the discovery of uncertain secondary findings, then it continues with a discussion with the patient and the family about the test results and their impact on both the direct care of patient and family members. For children, the genetic counselling on ethical concepts requires a clear,

accessible and informative communication with the patient and parents about the benefit/risk ratio and the rate of false positive results, which can involve a further investigations and false negative results, which might significantly delay the diagnostic and treatment. The decision to include the child in the discussion on genetic testing options is ultimately left to the parents or health care provider. The decision rests with the parents to make an informed decision regarding participation in the test, either by giving consent or by refusing, in the best interest of the child⁶.

Hallowell and the team used interviews in 2017 on treated FH children patients and their families who were genetic tested for the first time in their families' history. It was considered beneficial to understand the origin for their disease and to assess their own risk and their family members. Most parents decided for genetic testing and the children expressed their understanding of the performed tests, and most families did not report psychological issues due to FH diagnosis. Although there are unique benefits for the pediatric population, it is necessary to consider the special circumstances when testing a child for a condition in which the onset of the disease occurs in adult life, outside of the medical emergencies zone^{7,8}.

The American Academy of Pediatrics states that young people have an increasingly important role to play in their own health care decisions as they age and mature. From a legal perspective, there are no legal rights to ensure that a child has autonomy in the decision-making process of their own health care.

Understanding the psychological exposure for the child and the family and the confidentiality of the test results are essential ethical topics, and all potential risks must be considered and discussed before performing a genetic test in a child. Informed consent is mandatory to obtain from the parents, as the genetic information is complex and controversial issues of treatments of unknown efficacy add up to the difficulty of assessing the risks versus benefits of testing. The progress of genetic knowledge could lead to an uncertain exposure in maintaining confidentiality in the future, therefore it is required present rigorous monitoring of data recordings. Once a child has been tested, the results enter the medical record file. Since 2008 the Non-Discrimination of Genetic Information Act (GINA) defends people from health insurance discrimination based on genetic information when insurers request for life, disability or long-term care insurance, but it does not provide any guarantee that health insurers will pay for the genetic test or medical care that

a genetic test indicates as adequate medical necessity⁹.

Counseling for family planning and the necessary tests are also considered. Prenatal genetic testing presents a different set of risks and particular ethical considerations. Adults can choose and decide for themselves regarding testing in most cases, after medical-patient information.

The opposite situation is probable, when a supposedly healthy patient's parent requests the genetic testing. The requirement reflects an awareness of the risks involved in the investigation and the adjacent results. The medical responsibility is to reevaluate the benefit/risk ratio with the parents, including the child, when possible.

Situations of anxious or high emotional triggers need to consider psychological counselling in helping the family to better understand their own motivation when taking the decision, more likely to be more helpful than the testing itself, as genetic test results are diagnostic for life.

A person under 18th years old is not legally allowed to obtain genetic testing without parental permission, with rare exceptions. The 1998 World Health Organization's Proposed International Guidelines on Ethical Issues in Medical Genetics and the Provision of Genetic Services states: "Every genetic test should be offered in such a way that individuals and families are free to refuse or accept according to their wishes and moral beliefs. All testing should be preceded by adequate information about the purpose and possible outcomes of the test and potential choices that might arise. Children should only be tested when it is for the purpose of better medical care."

CONCLUSIONS

Are children capable in understanding the purpose and consequences associated with the results of short and long-term genetic testing?

First, our ethical and legal principles are autonomy, confidentiality, privacy, and equity as medical physicians. Based on them, we can understand each medical case individually, and decide when and what genetic tests to recommend based on the patient's needs.

Second, for the patient, the principle of autonomy implies the right to decide alone whether to perform genetic testing or not, without any constraint, and for the doctor the responsibility is to ensure before testing, orally and written, all necessary information on the procedure.

Third, specifically for a child patient it is difficult to

predict whether testing in childhood will be beneficial to that individual in adulthood. Some advocate genetic testing in suspected degenerative conditions where prevention is possible and may positively alter the affected individual's quality of life, as in the case of FH. It is thought that there are clear benefits in testing children, as atherosclerosis can be prevented with early identification and treatment, reducing the risk of cardiovascular disease.

The genetic testing progress has led to a paradigm shift in clinical practice, entering an era of prevention and personalized medicine. In the following years existing methods will become better understood, leading to clearer phenotype-genotype focus.

Compliance with ethics requirements:

The authors declare no conflict of interest regarding this article. The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law. Informed consent was obtained from all the patients included in the study.

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