Review

Cascade Screening in Familial Hypercholesterolemia: Advancing Forward

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Familial hypercholesterolemia is a genetic disorder associated with elevated LDL-cholesterol and high lifetime cardiovascular risk. Both clinical and molecular cascade screening programs have been implemented to increase early definition and treatment. In this systematic review, we discuss the main issues found in 65 different articles related to cascade screening and familial hypercholesterolemia, covering a range of topics including different types/strategies, considerations both positive and negative regarding cascade screening in general and associated with the different strategies, cost and coverage consideration, direct and indirect contact with patients, public policy around life insurance and doctor–patient confidentiality, the “right to know,” and public health concerns regarding familial hypercholesterolemia.


Key words: Familial Hypercholesterolemia, Cascade Screening, Genetic Cascade, Clinical Cascade, Cholesterol

Introduction

Familial hypercholesterolemia (FH) is a disorder characterized by the genetic predisposition to markedly higher LDL-c levels since birth due to an impaired clearance of circulating cholesterol¹⁻². FH is caused by mutations in genes encoding key proteins in the LDL receptor endocytic and recycling pathways causing elevation in plasma LDL cholesterol levels³. Mutations primarily in the LDLR but also APOB and PCSK9 genes are known genetic causes of FH³. This condition is of great concern because of the elevated cardiovascular risk and premature cardiovascular disease associated with a lifetime of high cholesterol in comparison to individuals with high cholesterol without FH whose cholesterol has gradually increased over time⁴.

A global concern over the identification and early treatment of this high-risk group has arisen in efforts to combat early cardiovascular disease and events and the financial cost burden they have on health systems⁴. Several different cascade programs have been developed across the world with varying success and have been analyzed for their effectiveness, coverage, and most importantly cost-effectiveness. While these cascade programs have advanced the area of FH research vastly due to its higher identification of patients, they have also identified several new considerations about the actual nature of the disease, which must be mitigated. Currently, we acknowledge that FH has a far greater complexity than once imagined with a polygenic influence over cholesterol levels, with the surrogate nature of LDL-c for cardiovascular disease now under closer scrutiny. Today, there are several issues related to the type of cascade screening that is the best fit for FH in its current state of flux of nomenclature, genetic particularities, risk classification, cost and issues surrounding patient rights, and quality of life.

In this systematic review, we discuss the main issues found in 65 different articles related to cascade screening and familial hypercholesterolemia to provide an overview of the current topics under consideration.
Methodology

This review used a keyword search of “cascade screening familial hypercholesterolemia” in the PubMed database, which retrieved 96 articles. These were further refined through an abstract review 26 eliminated (4-not in English, 2 repeats, 25 unrelated). After which 65 articles were read with themes delineated and integrated into a summary. Furthermore, original studies were derived from a bibliographic search of the reviews that were incorporated.

Resulting Summary

Of the 65 articles reviewed, the main themes found were as follows:
• Different types of cascade screenings programs;
• Guidelines and recommendations for appropriate and ethical implementations of FH cascade programs including public policy around life insurance and disclosure;
• Direct and indirect contact with family members; and
• Cost and coverage considerations.

2. Call for Cascade Screening

Cascade screening for FH can occur both clinically and genetically or using a hybrid model including both forms. Therefore, for cascade screening to occur, it must be effective in distinguishing between these two types of “high cholesterol” scenarios, the FH and non-FH patient. While for many adults the treatment will inevitably be based upon their phenotypic presentation and lipid-levels, the FH patient should lead to the identification of family members who carry the same genetic predisposition and elevated risk (having elevated LDL since birth). The target for public health is to reduce cardiovascular events/deaths before they occur by identifying those children and adolescents or young adults who would benefit from treatment as well as family members who are unaware of their elevated LDL as they have remained asymptomatic.

FH is one of the few diseases that has been reserved for genetic screening as a Centers for Disease Control and Prevention (CDC) Tier 1 Genomic Application5, 6) meeting all the criteria for all three categories of analytic validity, clinical validity, and clinical utility7). Based on the autosomal dominant inheritance patterns, recommendations support the use of cascade genetic screening to identify at-risk individuals7, 8). Unfortunately, there are several barriers that must be overcome until cascade genetic screening is widely implemented5, 9), as discussed below.

While experts agree that cascade screening should be implemented, there is no consensus as to the type of cascade screening program that is adequate or universally agreed upon. Current programs are critically evaluated and closely examined for effectiveness, false positive/negative rates, coverage, and cost effectiveness. Both selective and universal screenings methods are discussed below.

3. Infrastructure

Closely tied to cost-effectiveness cascade screening programs need an investment in infrastructure. Even the most basic program requires man-hours of personnel to identify index cases, conduct family tracing, perform testing, and evaluate and analyze the results. This precludes many from conducting cascade screening because of already overtaxed physician and medical staff, and additional personnel must either be hired or designated specifically (at least part-time) for this task. Several countries10-17) have organized centralized services that provide these services for a larger region; however, these programs require additional funding to support this infrastructure.

Implementation of a cascade screening requires several key elements (adapted from O’Kane et al, 201218):

I. Familial Hyperlipidemia Nurses and Staff

There are several tasks that require additional provisions for staffing. However, the array of tasks vary in their requisite training, ranging from making telephonic contact to genetic counseling, consideration should be taken in the most effective staffing decisions19) needed to cost effectively provide the necessary staff to support the program.

Both genetic and phenotypic approaches of screening require expertise in working with and giving advice to families20-24). Issues such as feeling isolated and anxious on learning about a familial mutation and/or receiving their own results have been reported by at-risk family members25). Globally, guidelines virtually recognize the need for specially trained personnel such as genetic counselors to assist in this aspect of cascade screening26, 27) to provide ethical treatment28).

II. Clinical Database

An FH database is necessary to allow documentation of family trees, screening status, and results in both index cases and family members. Intuitively, this step is obvious, though the importance of a well-organized database that allows for proper follow-up and contact when needed should be noted.
III. Index Cases
The initial identification of index cases is essential for cascade programs that conduct any type of screening. These index cases are often derived from a network of lipid clinics efficiently identifying FH in clinical practice.

IV. Familial Hyperlipidemia Molecular Diagnostic Service
(This applies only to cascade genetic screening program.) For DNA diagnosis to be undertaken a high-quality, accredited molecular genetics laboratory must champion the program as well as have personnel that is qualified in analysis and interpretation of the DNA results. Unfortunately, due to the extensive infrastructure needed for a cascade genetic screening there are only few such programs in the world.

4. Cost-Effectiveness
Multiple studies have been conducted on the varying cost-effectiveness of different screening approaches. While the individual results of these studies are beyond the scope of this review, most are well covered by Ademi et al. 29) in their review of economic evaluation of the detection and treatment of FH. Several additional studies 30) have since been performed; however, several of the same general criteria outlined in the above review should be applied while considering their implications.

In their review 29), they note that the cost-effectiveness of screening FH is dependent on the following three factors: i) cost-effectiveness of treatment, once FH is identified; ii) the underlying prevalence of FH among the screened population; and iii) the accuracy (validity) of the screening test. With regard to the first issue, the cost-effectiveness of treating FH is clear in known cases, but overall, the respective ICERs are highly sensitive to the costs of intervention. This, with the recent loss of exclusivity for high-potency statins brightens the horizon for screening programs on this front. On the other hand, screening introduces inefficiency at two levels. The first arises from the proportion of patients who are targeted for screening but who do not have the condition of interest - the efficiency of the screening strategy, and hence its cost effectiveness, is impaired. The second inefficiency arises from a lack of accuracy (validity) of the screening test. If the test is inaccurate because of low sensitivity, low specificity, or both, then the proportions of “false-positives” and “false negatives” will be high, leading to unnecessary and missed treatment, respectively.

Their review 29) identifies the following four current shortcomings in the applicability of cost effectiveness models for FH that have been conducted:

I. Models often use a sensitivity and specificity of 100% for genetic cascade screening, an assumption far from our current reality;
II. Models fail to factor in follow-up for patients without FH;
III. Future disease burden and hence the cost-effectiveness is underestimated as the underlying risk of coronary artery disease (CAD) is calculated from Framingham risk equations, an inadequate tool for this population 39);
IV. No account is made for those FH patients that are already undergoing treatment identified in cascade programs 29) who choose to not use pharmaceutical interventions 40).

5. Types of Cascade Program
I. Current Model: Selective screening
The current models of cascade screening utilized are considered “selective screening” in a family cascade approach. Selective screening targets a higher at-risk group resulting in a higher percentage of affected individuals identified (20%) 17) compared with a universal screening approach in the general population. Family cascade screening utilizes the inheritance pattern associated with autosomal dominant disease, where 50% of children are likely to inherit the disease. Thus a pedigree with an inheritance pattern helps to identify relatives to be targeted for screening. First-degree relatives (over the age of 2) are screened either by lipid profile and/or molecular testing for an identified pathogenic proband 41).

II. Clinical Cascade Programs
This model is sketched in Fig. 1A.
Clinical cascade programs intend to detect FH patients based on disease phenotype. The cascade follows the general principle of a patient initially diagnosed based on a selected clinical criterion. Often (but not always) at this point, they are referred to the cascade portion or a centralized program. Their family members are then traced and undergo cholesterol testing. As the index case has already been diagnosed with FH, having met other diagnostic criteria (such as family history or xanthomas), an elevated LDL-c level (usually >75th or 95th percentile) indicates they have the inherited condition.

III. Cascade Genetic Screening
This model is sketched in Fig. 1B.
Cascade genetic screening is used when an index case is identified as clinically defined FH, with some sort of clinical diagnostic criteria being used (Simon-
Broome or Dutch Lipid Clinic, MedPed). A genetic screening typically of LDLR, APOB, and PCSK9 are investigated searching for a pathogenic mutation. If a mutation is identified then that single-site mutation on the specific gene is searched for in family members (at a significantly reduced price) 50.

IV. Hybrid Model

This model is sketched in Fig. 1C.

This model incorporates both clinical and genetic screening. If a pathogenic mutation is found, the screening proceeds for a single-site variant in the family members. If a mutation is not found, their family members are still screened for cholesterol and evaluated by the clinical screening process.

Fig. 1 shows a basic schematic of all three models. Most genetic screening programs (Fig. 1B) often have several phases of screening: (1) first screening (2) multiplex ligation-dependent probe amplification (MLPA); still if no mutation is found, then a full exon analysis is conducted 50.

We have identified different points that are extensively discussed in the published literature reviewed. We elaborate these points below and have indicated them on the schematics as they impact the induced cascade model.

1. Recruitment of Index Cases: All selective screening programs have a difficulty in coverage of the general population 17, 42, 49. While primary index cases can be identified in hospitals or clinics, yet there remain limits in reaching the population as a whole 42, 44 (see universal screening).

2. Screening Region: There will be a certain loss in screening eligible family members who fall outside the screening area. The larger the area screened the lesser the program is affected. For smaller programs this is a severe limitation (losing more than 50% of first degree relatives) 16, 17 and often makes cascade screening infeasible for general practitioners as family members are often outside of their care 45. In many cases a centralized or national screening program is organized to encompass a larger region that receives referrals from smaller clinics 19.

3. Family contact (direct or indirect): Family contact is a prolific topic discussed in the literature as it touches upon the professional disclosure of familial genetic information 14, 46. Direct contact refers to medical personnel contacting family members through letters or telephone calls 46, 47 in contrast to index case disclosing their genetic status and recruiting relatives to participate in screening (indirect contact). The method chosen for contact (direct versus indirect) has been shown to drastically affect the success of cascade screening programs, with rates of “no response” of up to 60% 50 with indirect and 10% 13, 48 with direct 46. It is now widely accepted that the benefits of preventative and early treatment of FH warrants direct contact by screening programs 46, 49, 51.

4. Response to the call for screening of family members incorporates another point of exclusion for
those who have already been tested, those who are unable to be tested due to age or health status, and those who for various reasons prefer not to be screened, which can be over 40% of contacted family members depending on the program.

Several articles and guidelines have discussed concerns regarding genetic discrimination and public policy in guiding life insurers to fair practices. In a 2004 survey in the UK, utilizing a fictitious FH patient, life insurance premiums were reported 150% higher for those diagnosed with FH, which dropped down to 54% higher when treated, whereas another study found no current signs of genetic discrimination. For many countries, this foreshadows many of the impending issues that will need to be addressed with this new forefront.

5. Testing-Ambiguities and Discordance in current diagnostic criteria: Both clinical and molecular diagnostic criteria have limitations, which are much talked about themes in the area. We discussed briefly the main points reviewed.

Clinical Criteria

The selected clinical criteria may be Simon-Broome, Dutch Lipid Clinic, MedPed, or other selected criteria like the Japanese FH criteria. Each of the current clinical diagnostic criteria has its well-deserved critics as the understanding of FH continues to evolve. In reality, clinically diagnosing FH has a strong hurdle to overcome in distinguishing between the FH and non-FH patient. There is a wide overlap between the cholesterol levels of the individuals of FH and non-FH patients. In fact, the clinical phenotype between a heterozygote FH patient and a patient with high cholesterol from lifestyle factors is most often indistinguishable after a certain age and due to the numerous critics of using stigmata associated with diseases such as xanthomas and corneal arcus as delineating features of FH; many groups have adopted the more general criteria of LDL-c levels as the defining feature of FH. Unfortunately, cholesterol levels have some inherent ambiguities. As levels of cholesterol rise naturally over time, the older the patient is the progressively more difficult it is to distinguish FH from non-FH, causing a greater number of false-positives. Fluctuations of LDL-c levels during adolescence are also a cause of concern for screening programs utilizing solely cholesterol levels, as levels in males fall during puberty and rise as adulthood approaches, whereas in females they tend to rise in puberty. Given that, FH in children can often be ruled out if less than the 75th percentile, retesting LDL cholesterol levels at 18 years after the adolescent growth spurt is advised.

Molecular Test: There are several aspects that affect the reliability of the genetic test. FH genetics, like most other genetics programs, continues to advance at accelerated rates and continues to modify our understanding of not only the disease but also the complexity of the genetic component.

This method, while apparently the most “definitive” model is limited to the “comprehensiveness” of the search for the genetic mutation. Due to the laborious search of exon by exon screening there are often characteristic “time and cost saving measures” that allow this method to be more feasible, such as focusing on certain exons within the APOB gene or sequencing only the LDLR. Many programs work to genetically characterize their population using segregation or family studies initially, allowing for more selective searches for variants; however, these programs such as LIPOchip have met with only moderate sensitivity and specificity. While these cost and time saving measures are imperative for cost effectiveness consideration, they also cast a doubt on regions not studied. Many related studies point out that these measures cause an increasing number of false negative cases, suggesting the need for more comprehensive analysis.

In recent years, the landscape of genetic testing of FH has become more precarious in the notion that FH and elevations in LDL are a monogenic disorder. In fact, genetic mutations may not be detected in 20%-70% of patients in whom a clinical diagnosis of FH can be confidently made, requiring a significant proportion of family diagnostic testing to be carried out phenotypically. It has been through genetic programs in which the exploration of individuals who clearly exhibit phenotypic FH and a mutation is not found, which has expanded the understanding of LDL-c levels to be very polygenic, with mutations as well having variable penetrance in phenotype. The once considered trademark “high cholesterol” levels of FH caused by mutations within LDLR, APOB, or PCSK9 have in fact been found to be mitigated or exacerbated by a number of additional single nucleotide polymorphisms (SNPS) across several genes (polygenic FH). These polygenic influences alone (without any pathogenic mutation found in the classic 3 genes) can account for levels corresponding to homozygous FH (above 500 mg/dL). It has become clear that the genetics determining LDL-c levels is far more complex than originally believed with additional genes still being pursued. As such, failure to detect a mutation does not exclude a diagnosis of FH, particularly if the clinical phenotype is highly suggestive of FH. There are several rea-
VI. Child–Parent Screening Approach

In an effort to expand the reach of identifying index cases, universal screening of cholesterol levels in children has been proposed. As the cholesterol levels of newborns and adults are poor screening tests, a meta-analysis showed that between the ages of 1 and 9 years old a cholesterol test performs well as a screening tool for FH, as elevated levels of LDL would not be expected during these ages as lifestyle and environmental factors is a minimum. The screening test would detect 9 out of 10 cases with a low false positive rate (about one in 1,000). While 16–9 years old and 9–11 years old have also been proposed for a universal screening age, the feasibility of performing it at 2 years of age has some logistical benefits as it could be incorporated with immunization visits. From the identification of the child, the parents would then be screened, as a bottom up model, attempting to identify parents who may be asymptomatic. This has some added benefits, with those that would most benefit from early treatment clearly identified. This strategy

V. General Population Screening

Till date, no countries have reported on a universal screening program implemented; however, it remains a controversial topic with increasing evidence favoring a general population screening due to the low detection rates of selective screening programs. The aim of general lipid screening in childhood should be to find those with the highest lipid levels who will have the highest risk of developing premature coronary disease. The 2 most cited concerns regarding universal screening are cost and resource issues and the high number of false-positives that it would generate.

6. Complexities of Pathogenicity

Particular care needs to be taken when classifying gene variant as pathogenic, because it may be used in predictive testing of asymptomatic individuals. There is mounting evidence (along with a mounting number of mutation-positive phenotype-negative family members) of the questionable pathogenicity of the mutations listed in the international FH databases. A recent study evaluated a group of these patients and found that they had no greater elevated cardiovascular risk than normocholesterolemic mutation-negative patients and should not be considered for pharmaceutical intervention, clearly identifying these as false positives. And clearly, clinical management of the individual should be based on the plasma lipid phenotype (and other clinical criteria) and not on the genetic test results.

Beyond the scope of our review, we suggest you refer to the study of Benito-Vicente et al. (2015) on the integrated analysis required for familial hypercholesterolemia. In short, in silico analysis has failed to accurately predict the pathogenicity of the mutations (overestimating) reported in the FH database, leaving researchers struggling to test functionality. As such segregation studies or in-vivo functional analysis are needed with the lipid and molecular profiles of affected and non-affected relatives should be taken into account.

Fig. 2. Reasons for not finding a mutation in Subjects presenting the Familial Hypercholesterolemia Phenotype
Table 1. Difficulties encountered by the different cascade screening programs

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<th>Selective Screening Approaches</th>
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<td>Clinical Cascade</td>
<td>Genetic Cascade</td>
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<tr>
<td>1. Difficulties in Recruiting Index Cases</td>
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<td>2. False positives</td>
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<td>2.1 Ambiguities of clinical criteria</td>
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<td>2.2. Questionable pathogenicity</td>
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<td>3. False negatives</td>
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<td>3.1 Ambiguities of molecular criteria</td>
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<td>3.2 Polygenic forms</td>
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<td>4. Interpretation and family communication</td>
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is gaining support in Europe95).

Concerns were raised about general pediatric screening and the psychological impact of an FH diagnoses on children and parents20, 21, 96; however, evidence has yet to show serious detrimental effects of FH diagnoses in such cases97-101).

Clearly, a universal screening program would need to be tested and validated in a smaller population with these considerations in mind before it can be used on a more general basis39). Table 1 summarizes difficulties encountered by the different cascade screening programs.

6. Concluding Remarks

While a family-centric approach to screening promotes disease prevention and early diagnosis of heritable conditions on a population health level40), this method of identifying relatives of affected individuals has been criticized as it may only identify 50% of affected cases in the population; therefore, it does not have the coverage expected for a general screening program42). As there remains doubt with respect to the diagnoses, the ethical responsibilities of a cascade program must also mitigate the resulting false-positives and false-negatives that arise within each program.

The reality is that the genetic underpinnings of cardiovascular disease have been searched for, and we still remain distant from unraveling its complexity. While the genetics of cardiovascular disease remain out of our reach currently102), FH serves as a surrogate, with the defining features of this disease still in question. Therefore, a measure of long-term risk based on imaging of subclinical atherosclerosis may be more appropriate103) for treatment guidance.

Before we throw the baby out with the bath water, genetics programs are at the forefront of these ambiguities, often them being the ones to point them out. It is more complex that what we use now, this is universally agreed upon, how much more complex and will this complexity be able to be incorporated into an efficient cascade program that is not plagued with these doubts is yet to be seen. While the bath water may be muddy, it is important that these programs be applauded for moving our understanding and the field of research in FH forward by leaps and bounds and continues to study the cases that do not fit, as we imagine that the key to the complexity is how that they do fit.

References

3) Benn M, Watts GF, Tøbygaard-Hansen A, Nordestgaard BG: Familial hypercholesterolemia in the Danish general population: prevalence, coronary artery disease, and cho-
losterol-lowering medication. J Clin Endocrinol Metab, 2012; 97: 3956-3964
43) Hadfield GS, Humphries SE: Familial hypercholesterolaemia: Cascade testing is tried and tested and cost effective. BMJ, 2007; 335: 683
58) Daniels SR, Greer FR, the Committee on Nutrition: Lipid screening and cardiovascular health in childhood. Pediatrics, 2008; 122: 198-208
65) Huijgen R, Kindt I, Defesche JC, Kastelein JJ: Cardiovascular risk in relation to functionality of sequence variants in the gene coding for the low-density lipoprotein receptor: a study among 29,365 individuals tested for 64 specific low-density lipoprotein-receptor sequence vari-


82) Familial Hypercholesterolaemia Western Australia Program Committee. Model of care familial hypercholesterolaemia; 2008.


87) CLC-Bio. CLC Main Workbench. Aarhus, Denmark; 2011.

88) University College London: Low-density lipoprotein receptor familial hypercholesterolaemia database. London, United Kingdom; 2011 Available from: www.ucl.ac.uk/ldlr/Current/index.php?select db=LDLR


102) Versmissen J, Oosterveer DM, Yazdanpanah M, Deh-