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Expanded genetic testing in familial hypercholesterolemia—A single center's experience

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ABSTRACT

Objective: Assess the yield of genetic testing for pathogenic variants in *ABCG5, ABCG8, LIPA,* and *APOE* in individuals with personal and family histories suggestive of familial hypercholesterolemia.
Methods: Retrospective review of patients seen in the Advanced Lipid Disorders Clinic at Johns Hopkins
Results: In the lipid clinic at a single center during the years 2015–2023, 607 patients underwent genetic testing for familial hypercholesterolemia, of which 263 underwent the expanded genetic testing for sitosterolemia. Eighty-eight patients had genetic testing which included *APOE,* and 22 patients had testing which included *LIPA.* Among these, one patient was identified to have a pathogenic variant in *APOE* and another patient with a pathogenic variant in *ABCG5* (0.7 % yield). The frequency of a positive result was double that of a variant of uncertain significance.
Conclusion: These data suggest in rare cases expanded testing can provide answers for patients and families with a minimal likelihood of a variant of uncertain significance.

1. Introduction

Familial hypercholesterolemia (FH) is an autosomal semidominant hereditary dyslipidemia characterized by an elevated low-density lipoprotein cholesterol (LDL-C level) and premature coronary artery disease (CAD) [1,2]. An identifiable pathogenic variant has been reported in up to 70 % of cases [3-5]. The missing heritability is likely partially explained by a polygenic form and deep intronic variants [5-8]. Additionally, previous research has shown some patients presumed to have FH actually have a different hereditary dyslipidemia such as sitosterolemia, lysosomal acid lipase deficiency (LAL-D) or a pathogenic variant in *APOE* [9-11]. However, these phenocopies were only identified when additional genetic testing was pursued [5,9-11].

Correctly identifying patients with these rare dyslipidemias has important implications for medical management. Consequently, the American College of Cardiology recommends clinicians consider pursuing genetic testing for sitosterolemia, LAL-D, and *APOE* mutations in individuals with presumed FH where the genetic testing was negative [12]. In the case of sitosterolemia and LAL-D, treatment recommendations are different than the typical route for familial hypercholesterolemia. Because LDL-C levels are highly influenced by diet in sitosterolemia, restriction from plant sterol-rich foods is often the first-line strategy [13]. Furthermore, individuals with sitosterolemia typically do not respond well to statin therapy but do have a robust response to ezetimibe [14,15]. There is an enzyme replacement therapy specifically for LAL-D [16]. Unlike sitosterolemia and LAL-D, statin therapy is still the cornerstone therapy for patients with hyperlipidemia secondary to a pathogenic variant in *APOE.* However, it has been noted that *APOE* variant carriers may have a higher lipid lowering effect from statins suggesting a lower potency statin could be used in these patients [11,17]. Additionally, extracardiac findings are associated with both LAL-D and *APOE* variants indicating correct identification would provide clinicians with important information for management going forward. For example, some individuals with hyperlipidemia secondary to an *APOE* variant will also develop splenomegaly and thrombocytopenia [18]; while liver dysfunction, splenomegaly, platelet disorder, and persistent diarrhea are associated with LAL-D [19]. Lastly, correct identification of these rare disorders provides information regarding CAD risk [19-21].

Accurate identification of these phenocopies also provides important

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information for familial screening. Both LAL-D and sitosterolemia are autosomal recessive conditions [2]. Of note, a clinical phenotype of hyperlipidemia and increased coronary artery disease risk has been reported for individuals with a single *ABCG5* loss-of-function mutation [21]. Additionally, individuals who are heterozygous for a pathogenic variant in *ABCG8* or *ABCG5* may have an exaggerated response to dietary reduction of sterols [22]. Hyperlipidemia secondary to a pathogenic *APOE* variant is inherited in an autosomal dominant pattern, but there is a recessive form reported called sea-blue histiocytosis [23].

Based on this information, the American College of Cardiology recommends clinicians consider an expanded genetic testing approach in cases with presumed FH where genetic testing was negative to assess for these rare dyslipidemias [12]. Previous groups have reported incidence rates or case reports for individual genes [5,9,10], but no previous report has looked at the utility of reflexing to a panel of genes which can mimic FH. Given this approach is being increasingly offered by genetic testing laboratories, we performed a retrospective review to try assess the clinical utility of an expanded panel.

2. Methods

A retrospective review was conducted of patients seen in the Advanced Lipid Disorders Clinic at Johns Hopkins University from 2015 to 2023. The research protocol was approved by the Institutional Review Board at Johns Hopkins University. Individuals with both a history suggestive of familial hypercholesterolemia and who underwent genetic testing were included in the study. If multiple lipid panels were available, the panel with the highest LDL-C level was used in analysis. If untreated lipid levels were unavailable, LDL-C and triglyceride levels were estimated based on the average lipid-lowering reduction of the patient's medications at the time of analysis [24]. To calculate the total cholesterol, the Friedewald equation was rearranged and subsequently applied [25]. HDL-C was assumed to be the same. Physical exams were performed by cardiologists with expertise in FH and included evaluation for tendon xanthomas and corneal arcus, the latter considered a physical finding of FH only if present before age 45. Family histories were obtained by a genetic counselor.

Genetic testing was performed on blood/buccal samples at the following CLIA certified laboratories: Invitae, GeneDx, Ambry, and GBinsight. Which laboratory was used typically depended on the patient's insurance. Ina few cases patients had genetic testing previously done by the referring provider. All patients underwent sequencing and deletion/duplication analysis of four genes associated with familial hypercholesterolemia (*LDLR, PCSK9, APOB,* and *LDLRAP1*). After publication of the American College of Cardiology statement recommending consideration of expanded testing in 2018, we began to offer the option of reflex testing to patients whose original results were negative or uncertain. This originally just included *ABCG5, ABCG8,* and *LIPA* due to lab offerings. Reflex was expanded to include *APOE* beginning in 2022 which had previously not been routinely included due to lab offerings (Fig. 1). Genetic testing for LAL-D was limited to patients who had concomitant hyperlipidemia and unexplained elevated liver enzymes/liver disease based on the diagnostic algorithm proposed by Reiner et al. [26] and the lack of evidence for patients presenting with isolated hyperlipidemia [27]. Whether patients proceeded with the reflex testing depended on insurance coverage and patient preference. The *APOE* genotypes were not reported unless the provider had ordered testing through GBinsight due to most clinical genetic testing laboratories not reporting the genotype in accordance to the American College of Medical Genetics Statement regarding testing for *APOE* [28]. Variants were classified according to the 2015 American College of Medical Genetics guidelines [29]. Classifications were based on genetic information available on each variant in 2023.

Statistical differences between study groups were assessed using the Mann–Whitney test. A p-value <0.05 was considered statistically significant.

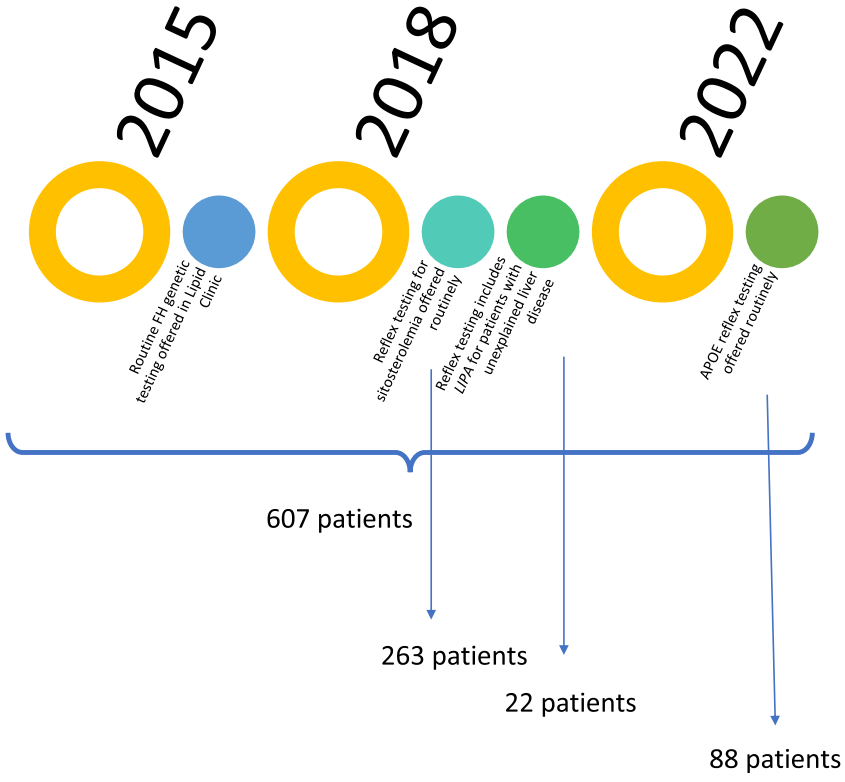

Fig. 1. Flowchart participant inclusion and results.

3. Results

Between 2015 and 2023, 607 patients underwent genetic testing for FH at the Advanced Lipids Disorders Clinic of the Johns Hopkins Hospital. Patients who only underwent genetic testing for the four main FH genes ($n = 280$) were excluded. An additional 64 patients who received a positive genetic test results for FH ($n = 64$) were excluded because the genetic test did not then reflex to the additional genes. The remaining 263 patients had sequencing and duplication/deletion analysis for the genes associated with sitosterolemia (*ABCG5* and *ABCG8*). Additionally, 88 patients of these patients also had analysis of *APOE,* and 22 patients with either elevated liver enzymes or nonalcoholic fatty liver disease had genetic analysis of *LIPA* for lysosomal acid lipase deficiency (Fig. 2a).

Participant demographics are listed in Table 1. As would be expected, patients had a history of hyperlipidemia (mean LDL-C 212 ± 53 mg/dL) and the vast majority had a family history of hyperlipidemia and/or premature CAD (89 %). Lipoprotein (a) concentration was available on 216 patients. The concentration ranged from <8.4–600 nmol/L (median 74 nmol/L, IQR 26–180 nmol/L). Only a small proportion of individuals had physical sequalae suggestive of FH, but it should be noted 22 % ($n = 59$) were only seen by telemedicine due to the COVID-19 pandemic. The mean Dutch Lipid Clinic Network (DLCN) Score was 5 indicating more than half of patients had only "possible FH" [30]. The median DLCN Score was 4. There was a high proportion of women (64 %) and individuals of White ancestry (64 %).

Expanding testing to include *ABCG5* and *ABCG8* did not identify any patients with sitosterolemia. It did identify one individual with a pathogenic truncation in *ABCG5* (c.575delG, p.G192Afs*35). This individual had a history of hyperlipidemia (LDL-C 276 mg/dL) along with elevated liver enzymes which had been attributed to supplement use. The patient was started ezetimibe but had an adverse reaction to the

Table 1
Demographics of participants.

Race	*n* (%)
White	170 (64)
Black	35 (35)
Asian	29 (11)
Ashkenazi Jewish	20 (8)
Latino	9 (3.7)
Native American	1 (0.3)
Gender	
Male	96 (36)
Female	168 (64)
Age at time of appointment (years)	48 ± 16
Lipid profile	
Total cholesterol (mg/dL)	296 ± 59
Triglycerides (mg/dL)	138 ± 70
HDL (mg/dL)	59 ± 26
LDL-C (mg/dL)	212 ± 53
Median Lp(a) (nmol/L)	74
Physical exam	
Xanthomas	17 (6.4)
Corneal arcus (<45 years old)	1 (0.3)
Premature coronary artery disease	60 (23)
Positive family history	236 (89)
Mean Dutch lipid clinic network score	5

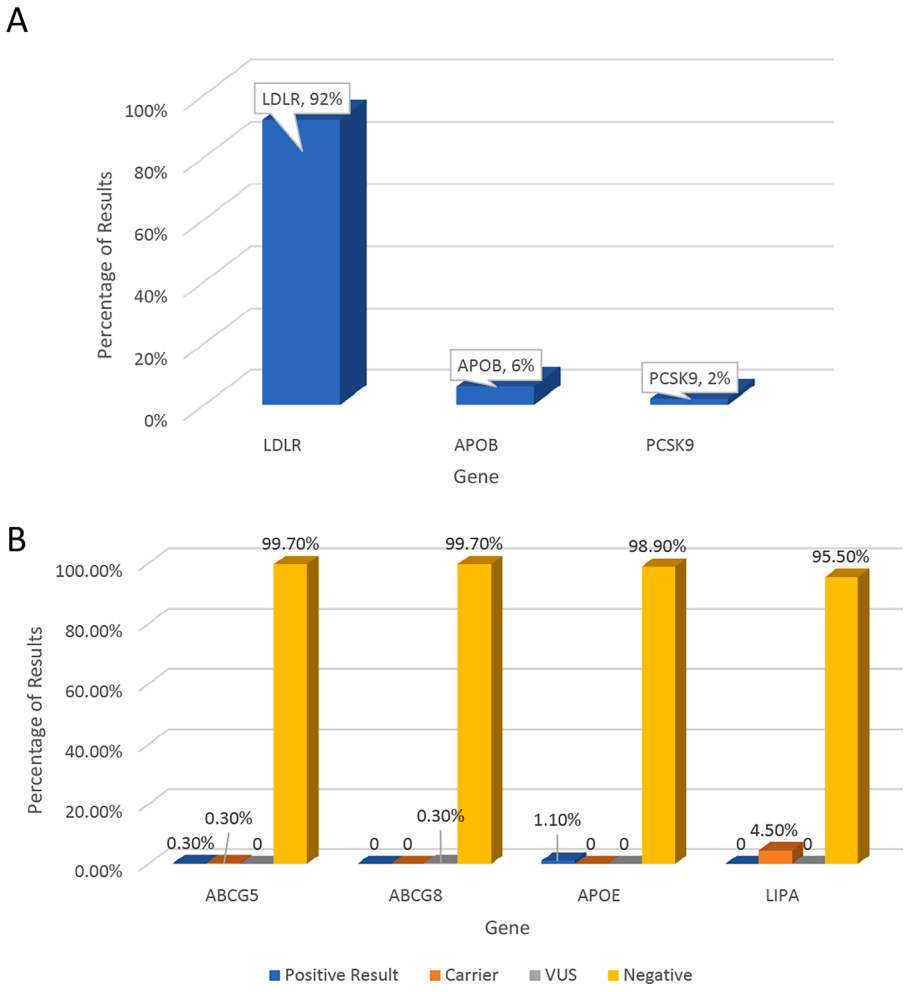

Fig. 2. Yield of genetic testing. A) Yield for main FH genes. B) Yield when incorporating additional genes which mimic FH.

Table 2
Clinical details of patients with pathogenic and variants in expanded panel genes.

ID	Age at initial visit	Race	Biological Sex	Genetic Results	Variant	TC (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	Lp(a) (nmol/L)	Family history	Physical findings	Premature CAD	Liver disease
3821	50	White	Female	Pathogenic	APOE c.500_502delTCC (p.Leu167del)	554	91	88	445	52	Yes	No	Yes	Fatty liver disease
3722	33	White	Male	Pathogenic	ABCG5: c.575delG, p. G192Afs*35	347	149	41	276	24	Yes	no	No	Elevated liver enzymes
3751	54	White	Female	VUS	ABCG8: c.1703A>C, p. Asn568Thr; LDLR: c.1156G>T, p. Asp386Tyr	398	185	65	247	235	Yes	No but telemed	Yes	No

medication limiting ability to assess response. One VUS was identified in one individual in *ABCG8* (c.1703A > C, p.Asn568Thr). The clinical details are summarized in Table 2. Overall, the yield was 0.38 %.

One patient out of the 88 (1.1 %) who underwent genetic testing for *APOE* was identified to have a pathogenic, inframe deletion (c.500_502delTCC, p.Leu167del). She had a history of pure hypercholesterolemia (LDL-C 445 mg/dL and triglycerides 91 mg/dL) along with hepatosplenomegaly and steatosis. (Table 2). No VUSes were identified in *APOE* for any patients.

No patients with LAL-D were diagnosed. Of the 22 patients whose genetic testing include *LIPA,* one carrier was identified. No VUSes were reported in *LIPA*.

Variants of uncertain significance were rare. A VUS in these four additional genes was identified in only one individual (0.35 %). This VUS rate is similar to the rate seen for the standard four gene panel. There was not a statistical difference in the frequency of a VUS between the two groups ($p = 0.94$).

4. Discussion

The overall yield of genetic testing for FH within our population (20 %) was consistent with other centers' yield when pursuing testing for individuals who do not already meet or score highly using the DLCN criteria [31]. We elect to use a more aggressive approach in terms of genetic testing because some patients will not score highly on the DLCN criteria despite having FH for a variety of reasons such as long-term statin use, young age at diagnosis, and being adopted [32].

Incorporating reflex genetic testing for sitosterolemia did increase the genetic testing yield, albeit slightly which is consistent with findings from other groups [10,11,21]. Nomura et al. found the frequency of loss of function *ABCG5* variants was 0.12 % in individuals from the UK Biobank with CAD [21]. Notably we did not identify any patients with sitosterolemia. This may be in part due to the fact that the majority of our patients were adults. Another study identified an individual with sitosterolemia, but this was a pediatric patient [5]. Additionally, the majority of our patients had a least one first-degree relative with either hyperlipidemia or premature CAD, and given sitosterolemia is a recessive condition this might also partly have contributed to the low detection rate [2].

While our cohort numbers are small for *APOE* and *LIPA,* reflex testing did identify the genetic etiology for one additional patient. This is consistent with other reported cohorts for *APOE* which has ranged from 0.3 to 3 % [10,11]. Similar to sitosterolemia, the lower yield for LAL-D may have been partly influenced by the fact that the majority of our patients were adults with a positive family history [2,5].

Of note, increasing the number of genes tested did not significantly increase the VUS rate. Previous publications have argued against increasing genetic testing panels due to the concern that uncertain results have been associated with increased patient anxiety, frustration and decisional regret [33-35]. This concern may not need be the case for dyslipidemias, at least when the genes are still targeted to a specific phenotype.

There are limitations with the study that need to be acknowledged. A main limitation is study size especially for the number of patients tested for LAL-D and *APOE.* Additionally, the majority of our cohort is of White ancestry, and this may have impacted genetic testing yield. Finally, this is representative of a single-center experience.

Overall, we were able to provide genetic answers to two additional patients and their families allowing for cascade screening. This was done with minimal risk for a VUS. In fact, the likelihood of a positive result was double the likelihood of a VUS. Thus, we conclude that expanded testing in patients with presumed FH who test negative for the four main genes could be considered, although the yield is low, given positive results may lead to changes in medical management

Statement of ethics

The present study was conducted in accordance with the Declaration of Helsinki and was also approved by the Institutional Review Board of Johns Hopkins University School of Medicine.

Sources of funding

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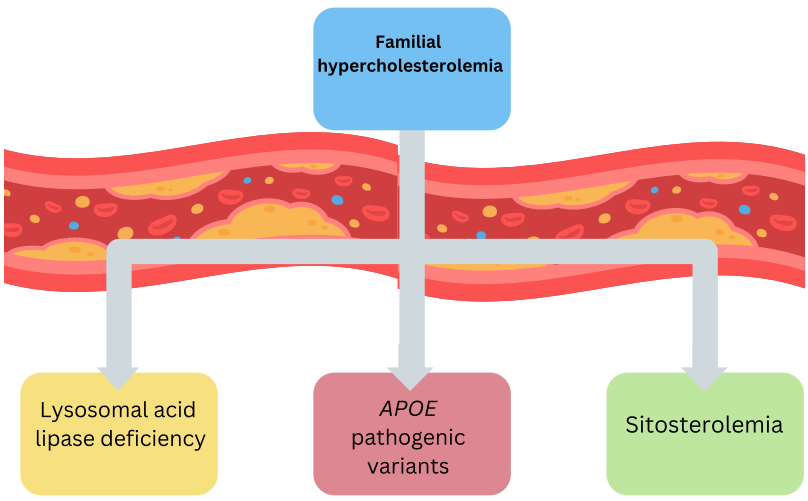

Central illustration

CRediT authorship contribution statement

Emily E. Brown: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Conceptualization. **Kathleen Byrne:** Writing – review & editing. **Erin D. Michos:** Writing – review & editing, Conceptualization. **Thorsten M. Leucker:** Writing – review & editing. **Francoise Marvel:** Writing – review & editing. **Steven R. Jones:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Seth S. Martin:** Writing – review & editing. **Marios Arvanitis:** Writing – review & editing, Validation, Supervision, Methodology, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Emily Brown reports a relationship with Novartis Pharmaceuticals Corporation that includes: speaking and lecture fees. Seth Martin reports a relationship with Novartis Pharmaceuticals Corporation that includes: consulting or advisory. Seth Martin reports a relationship with Amgen Inc. that includes: consulting or advisory. Seth Martin reports a relationship with Merck & Co Inc. that includes: consulting or advisory. Seth Martin reports a relationship with AstraZeneca Pharmaceuticals LP that includes: consulting or advisory. Seth Martin reports a relationship with Novo Nordisk Inc. that includes: consulting or advisory. Seth Martin reports a relationship with Sanofi that includes: consulting or advisory. Seth Martin reports a relationship with Kaneka Corporation that includes: consulting or advisory. Erin Michos reports a relationship with Amgen Inc. that includes: consulting or advisory. Erin Michos reports a relationship with Amarin Pharma Inc. that includes: consulting or advisory. Erin Michos reports a relationship with AstraZeneca Pharmaceuticals LP that includes: consulting or advisory. Erin Michos reports a relationship with Bayer Corporation that includes: consulting or advisory. Erin Michos reports a relationship with Boehringer Ingelheim Corp USA that includes: consulting or advisory. Erin Michos reports a relationship with Edwards Lifesciences Corporation that includes: consulting or advisory. Erin Michos reports a relationship with Medtronic Inc. that includes: consulting or advisory. Erin Michos reports a relationship with Novartis Pharmaceuticals Corporation that includes: consulting or advisory. Erin Michos reports a relationship with Novo Nordisk Inc. that includes: consulting or advisory. Erin Michos reports a relationship with Pfizer that includes: consulting or advisory. Erin Michos reports a relationship with Esperion Therapeutics Inc. that includes: consulting or advisory. Editor-in-Chief AJPC-EM.

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