Limited-Variant Screening vs Comprehensive Genetic Testing for Familial Hypercholesterolemia Diagnosis

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IMPORTANCE Familial hypercholesterolemia (FH) is the most common inherited cardiovascular disease and carries significant morbidity and mortality risks. Genetic testing can identify affected individuals, but some array-based assays screen only a small subset of known pathogenic variants.

OBJECTIVE To identify the number of clinically significant variants associated with FH that would be missed by an array-based, limited-variant screen when compared with next-generation sequencing (NGS)-based comprehensive testing.

DESIGN, SETTING, AND PARTICIPANTS This cross-sectional study compared comprehensive genetic test results for clinically significant variants associated with FH with results for a subset of 24 variants screened by a limited-variant array. Data were deidentified next-generation sequencing results from indication-based or proactive gene panels. Individuals receiving next-generation sequencing-based genetic testing, either for an FH indication between November 2015 and June 2020 or as proactive health screening between February 2016 and June 2020 were included. Ancestry was reported by clinicians who could select from preset options or enter free text on the test requisition form.

MAIN OUTCOMES AND MEASURES Number of pathogenic or likely pathogenic (P/LP) variants identified.

RESULTS This study included 4563 individuals who were referred for FH diagnostic testing and 6482 individuals who received next-generation sequencing of FH-associated genes as part of a proactive genetic test. Among individuals in the indication cohort, the median (interquartile range) age at testing was 49 (32-61) years, 55.4% (2528 of 4563) were female, and 63.6% (2902 of 4563) were self-reported White/Caucasian. In the indication cohort, the positive detection rate would have been 8.4% (382 of 4563) for a limited-variant screen compared with the 27.0% (1230 of 4563) observed with the next-generation sequencing-based comprehensive test. As a result, 68.9% (848 of 1230) of individuals with a P/LP finding in an FH-associated gene would have been missed by the limited screen. The potential for missed findings in the indication cohort varied by ancestry; among individuals with a P/LP finding, 93.7% (59 of 63) of self-reported Black/African American individuals and 84.7% (122 of 144) of Hispanic individuals would have been missed by the limited-variant screen, compared with 33.3% (4 of 12) of Ashkenazi Jewish individuals. In the proactive cohort, the prevalence of clinically significant FH variants was approximately 1:191 per the comprehensive test, and 61.8% (21 of 34) of individuals with an FH-associated P/LP finding would have been missed by a limited-variant screen.

CONCLUSIONS AND RELEVANCE Limited-variant screens may falsely reassure the majority of individuals at risk for FH that they do not carry a disease-causing variant, especially individuals of self-reported Black/African American and Hispanic ancestry.

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Familial hypercholesterolemia (FH) is the most common inherited cardiovascular disease, with an estimated prevalence of 1:176 to 1:282. FH causes lifelong exposure to elevated levels of low-density lipoprotein (LDL) cholesterol that can lead to premature coronary artery disease and early death, exceeding the risks among patients with equivalent levels of LDL cholesterol due to multiple alternative factors. Timely pharmacological interventions can significantly reduce morbidity and mortality risks, but unfortunately most individuals with FH are undiagnosed and thus untreated. FH can be diagnosed clinically and via genetic testing, with the latter offering several advantages including the ability to identify affected individuals who do not meet specific cholesterol-level thresholds as well as asymptomatic and presymptomatic relatives who may be at risk for the specific disease-causing variant identified in the family.

However, genetic tests vary greatly in their quality and in how many of the thousands of known and novel FH-associated genetic variants they include.

FH is an autosomal dominant disorder most frequently associated with variants in the LDL receptor (LDLR) gene. Less frequently, FH is caused by variants in the apolipoprotein B (APOB) gene, primarily in the LDLR-binding region of the protein, or by gain-of-function variants in proprotein convertase subtilisin/kexin 9 (PCSK9). Additionally, a rare, recessive form of FH is caused by variants in the LDLR adaptor protein 1 gene (LDLRAP1). The particular gene affected and the type of variant can influence an individual’s level of LDL cholesterol and risk for developing coronary artery disease, as well as the intensity of pharmacologic intervention needed to control cholesterol levels. LDLR null variants are most severe, while individuals with other LDLR variants, APOB variants, and PCSK9 variants generally have a milder phenotype.

Genetic tests for FH are available in clinical settings, where a clinician will order the test on behalf of a patient, and in consumer settings, where an individual may order a test directly from a testing laboratory (often called direct-to-consumer testing). In both settings, genetic tests frequently use either array-based genotyping or next-generation sequencing (NGS) to detect variants. Critically, the technology used in a genetic test can substantially affect clinical utility. Array-based genotyping analyzes a limited set of genetic positions already known to have disease-causing variants. Sequencing-based testing is more comprehensive because it can analyze full gene sequences and detect not only known but also novel and private variants. One array-based product offered by a health and ancestry company focused on direct-to-consumer testing screens only 24 FH-associated variants as of July 2020, whereas tests that use NGS can detect more than 2000 known FH-associated variants as well as novel clinically actionable (pathogenic or likely pathogenic [P/LP]) variants.

Although most limited-variant screens are not offered in a medical or diagnostic setting, consumers may look to the results for guidance on health behaviors. Negative findings on a limited-variant screen in an individual who actually carries a disease-causing variant may lead to false reassurance that a disease-associated variant is not present, resulting in missed opportunities to initiate or intensify treatment and identify at-risk relatives. In addition, some limited-variant screens do not report whether an individual harbors 1 or 2 copies of a variant, whereas this is routine for NGS-based testing and can influence clinical management and outreach to relatives who may also need testing. Direct comparisons of these methods are needed to demonstrate how many patients with disease-associated FH variants might be identified with NGS but remain unidentified by limited-variant screens.

Here, we performed retrospective analyses of NGS results from individuals referred for FH diagnostic testing and individuals who received NGS of FH-associated genes as part of a proactive genetic test. We determined how many individuals with positive findings of FH variants on NGS would have been missed by a limited, 24-variant screen.

**Methods**

We reviewed deidentified data under an institutional review board–approved protocol (Western Institutional Review Board #20161796) for 2 cohorts of unrelated individuals: individuals referred by an ordering clinician for FH genetic testing whose samples were received from November 2015 through June 2020 and individuals whose samples for proactive genetic testing were received from February 2016 through June 2020. Patient consent for genetic testing was obtained by the ordering clinician, who attested to this on the requisition form at the time of ordering. The proactive panel (Invitae Genetic Health Screen) was either ordered by a clinician on behalf of an individual or a request for a test was initiated by an individual with subsequent third-party physician approval. The proactive panel included up to 147 genes addressing a broad range of inherited health conditions, including FH. To reduce potential bias toward individuals with existing cardiological health concerns, we excluded individuals who ordered the cardiology-focused proactive test (Invitae Cardio Screen). Age and ancestry (selected from preset options or entered as free text on the test requisition form) were determined by self-report (from the tested individual or their clinician); individuals with missing ancestry data were grouped as unknown in ancestry-stratified analyses. Both cohorts were tested for more than 2000 possible variants (single-nucleotide variants, insertions/
deletions, and copy number variants) in 4 FH-associated genes: \(LDLR\), \(APOB\), \(PCSK9\), and \(LDLRAP1\). Because loss-of-function variants in \(APOB\) are associated with familial hyperbetalipoproteinemia (a clinically distinct condition from FH), 12 loss-of-function \(APOB\) variants (none of which would be reported by the partial screen) were excluded from the analyses.

Our projection of the limited-variant comparison assay was guided by a package insert, last updated in August 2020, which lists 24 specific single-nucleotide variants for the FH assay: 23 variants in \(LDLR\) and 1 in \(APOB\). Per a February 2019 statement from the manufacturer of the comparison assay, our analysis assumed that the limited-variant assay does not report whether an individual is heterozygous or homozygous for a variant.14

### Gene Sequencing and Variant Interpretation

Sequencing and variant classification were as previously described.15,16 Briefly, genes were targeted with oligonucleotide baits (Agilent Technologies, Roche, and Integrated DNA Technologies) to capture the exons ±10 base pairs of adjacent intronic sequence and certain noncoding regions of clinical interest. Targeted regions were sequenced to a mean depth of 350× read coverage (minimum 50×). All primary sequencing was performed on Illumina HiSeq or NovaSeq instruments (Illumina). Sequencing reads were aligned using NovoAlign (Novacraft), followed by single-nucleotide variant and small insertions/deletions variant identification using a modified GATK haplotype caller. Break points, exon-level copy number variants, and variants within homopolymers were called with additional methods.17 Resulting variants were assigned to 1 of 5 categories based on a score-based refinement of the 2015 sequence variant interpretation guidelines from the American College of Medical Genetics and Genomics and the Association for Medical Pathology: benign, likely benign, uncertain significance, likely pathogenic, or pathogenic.16,18 Of 382 unique disease-associated variants included in these analyses, 322 (84.3%) were classified as pathogenic and 60 (15.7%) were classified as likely pathogenic (eTable 1 in the Supplement).

We grouped individuals with P/LP findings into 4 groups: (1) heterozygous FH for individuals with a single P/LP variant in \(LDLR\), \(APOB\), or \(PCSK9\); (2) homozygous FH for individuals with homozygous or compound-heterozygous P/LP variants in \(LDLR\), \(APOB\), or \(PCSK9\); (3) autosomal recessive hypercholesterolemia for individuals with 2 P/LP variants (homozygous or compound-heterozygous) in \(LDLRAP1\); and (4) autosomal recessive hypercholesterolemia carrier for individuals with a single P/LP variant in \(LDLRAP1\). Positive detection rate was defined as the proportion of individuals with heterozygous FH, homozygous FH, and autosomal recessive hypercholesterolemia among all individuals in each cohort.

### Cascade Analysis

To compare the outcomes of cascade screening (testing of family members of affected individuals), we reviewed existing records for relatives of individuals with a P/LP result. Relatives who had testing results at the variant or gene level were grouped by proband (the individual initially included in the cohort) and assessed for the relevant FH-associated variant(s).

### Statistical Analysis

The prop.test function in statistical software package R version 3.3.0 (R Foundation for Statistical Computing) was used to determine differences in positive detection rates between comprehensive NGS and the limited-variant screen for both the indication and proactive cohorts. Significance was set at \(P < .005\).

### Results

#### Diagnostic Testing vs Limited-Variant Screen

The indication cohort included 4563 individuals referred by health care professionals for FH testing based on clinical suspicion. The median (interquartile range) age at testing was 49 (32-61) years, and 2528 individuals (55.4%) were female (Table 1). By self-report, 2902 (63.6%) were White/Caucasian, 315 (6.9%) were Hispanic, 280 (6.1%) were Black/African American, and 214 (4.7%) were Asian (Table 1).

The positive detection rate by NGS was 27.0% (1230 of 4563) (eTable 2 in the Supplement). In contrast, if these individuals had been tested using the limited 24-variant array-based test, only 8.4% (382 of 4563) would have been identified and informed that they carry a disease-associated variant. Thus, 69% (848 of 1230) of individuals with P/LP variants identified in the NGS-based test would have been missed by the array-based test (Figure 1; eTable 2 in the Supplement). The performance of the limited-variant screen compared with the comprehensive test varied widely by race and ethnicity (Figure 2). The limited-variant screen would have missed

### Table 1. Demographic Characteristics of Indication and Proactive Cohorts

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. (%)</th>
<th>Proactive cohort (n = 6482)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at testing, y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18-50</td>
<td>1696 (37.2)</td>
<td>3544 (53.3)</td>
</tr>
<tr>
<td>&gt;50</td>
<td>2188 (48.0)</td>
<td>3028 (46.7)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>2035 (44.6)</td>
<td>3298 (50.9)</td>
</tr>
<tr>
<td>Female</td>
<td>2528 (55.4)</td>
<td>3185 (49.1)</td>
</tr>
<tr>
<td>Self-reported ancestry</td>
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<td></td>
</tr>
<tr>
<td>Ashkenazi Jewish</td>
<td>72 (1.6)</td>
<td>224 (3.5)</td>
</tr>
<tr>
<td>Asian</td>
<td>214 (4.7)</td>
<td>354 (5.5)</td>
</tr>
<tr>
<td>Black/African American</td>
<td>280 (6.1)</td>
<td>74 (1.1)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>315 (6.9)</td>
<td>106 (1.6)</td>
</tr>
<tr>
<td>Multiplea</td>
<td>132 (2.9)</td>
<td>406 (6.3)</td>
</tr>
<tr>
<td>Otherb</td>
<td>201 (4.4)</td>
<td>404 (6.2)</td>
</tr>
<tr>
<td>Unknown</td>
<td>447 (9.8)</td>
<td>906 (14.0)</td>
</tr>
<tr>
<td>White/Caucasian</td>
<td>2902 (63.6)</td>
<td>4010 (61.9)</td>
</tr>
</tbody>
</table>

\(P < .005\).

*a Multiple includes 1 or more combinations of the ancestry groups listed in the Table and under Other.

*b Other includes French Canadian, Mediterranean, Native American, Pacific Islander, and Sephardic Jewish.
33% (4 of 12) of Ashkenazi Jewish individuals, 64% (420 of 661) of White/Caucasian individuals, 85% (122 of 144) of Hispanic individuals, and 94% (59 of 63) of Black/African American individuals positive for FH-associated variants (Figure 2).

Additionally, NGS-based testing revealed clinically important aspects of FH genetics that may have been missed by limited-variant screening. This included 132 P/LP copy number variants that were detected by NGS in LDLR and accounted for 10.7% (132 of 1230) of the positive detection rate. Second, while the comprehensive test identified 4 individuals with a single P/LP variant in LDLRAP1 (autosomal recessive hypercholesterolemia carriers) and 9 individuals with a P/LP variant in PCSK9 (homozygous FH), none would have been identified by the limited-variant screen because LDLRAP1 and PCSK9 are not included in the limited-variant assay (Table 2). Third, the comprehensive test identified 74 individuals with homozygous FH (Table 2). Although 37 of the individuals with homozygous FH would have received a P/LP finding from the limited-variant screen, by our analysis none would have been informed of their severe homozygous or compound-heterozygous state.

Cascade Screening

Given the importance of cascade (family) screening for identifying additional individuals with FH, we evaluated the uptake of cascade testing in the indication cohort and assessed the possible association of missing P/LP variants with outcomes if the limited-variant screen had been used. Among 1230 individuals with P/LP findings, 256 (20.8%) had at least 1 family member who was tested for the P/LP variant (Table 3). As a result of cascade testing, 550 family members were tested, of whom 321 individuals (58.4%) had a P/LP finding. With the limited-variant screen, only 178 additional individuals would have been tested, of whom 112 (62.9%) would have had a P/LP finding (Table 3). Thus, 65.1% of additional individuals with a P/LP FH variant would have been missed.

Proactive Testing vs Limited-Variant Screen

We also examined FH yields in individuals who had proactive genetic testing. Among 6482 individuals, 34 (0.5%) were found to carry at least 1 disease-associated variant in an FH-associated gene (eTable 2 in the Supplement), corresponding with a population prevalence of approximately 1:191. Only 13 of these individuals would have received findings of a P/LP variant with the limited-variant screen, indicating that 21 of the 34 individuals (61.8%) with a P/LP variant would have been missed (Figure 1). Finally, of 39 positive probands in the proactive cohort, only 2 had family members who were also tested for FH-associated genes (Table 3). Only 1 of these 2 families would have been alerted to their need for testing had the proband received results from the limited-variant screen.

Discussion

This study demonstrates that comprehensive NGS-based genetic testing identifies substantially more individuals affected by FH than a limited-variant screen. These results have significant implications for individuals with FH, their families, and cardiologists and other clinicians. The results suggest that two-thirds of people with a P/LP variant in a gene associated with FH would receive a negative result from a limited-variant screen that is marketed as a report of the genetic health risk for FH.14 The inferior detection rate of the limited-variant screening was most pronounced among Hispanic and Black/African American individuals. The reduced yield of limited-variant screening could result in a major health care disparity for groups already affected by social and medical disenfranchisement that beget serious health disparities including a significantly higher rate of cardiovascular death among Black/African American individuals.19

Missed opportunities to identify individuals affected by FH could affect a relatively large portion of the population, as we found that approximately 1:191 individuals (0.52%) in the ostensibly healthy proactive cohort carried at least 1 P/LP FH variant. This prevalence estimate is nearly identical to the population prevalence of incidental findings for hereditary lipid disorders (0.5%) in a study of more than 20 000 individuals.20 In contrast with NGS-based genetic testing, the limited-variant screen evaluated in this study addresses only 24 out of thousands of potential disease-causing variants in FH-associated genes.11,13,14 does not report whether an individual harbors 1 or 2 copies of a variant,13,14 and does not detect copy number variants, which are reported to cause approximately 10% of FH cases.21 It should be noted that ancestry-focused direct-to-consumer tests have reportedly been used by more than 26 million individuals.22 If FH screening were part of the major-

![Figure 1. Percentage of Individuals With Clinically Significant Familial Hypercholesterolemia Variants as Detected by a Comprehensive Test and a Limited-Variant Screen](https://jamanetwork.com/)

Positive detection rate (%) in the familial hypercholesterolemia indication cohort (n = 4563) and the proactive cohort (n = 6482) as detected by comprehensive gene sequencing (comprehensive test) and projected for 24 familial hypercholesterolemia variants included in a limited-variant screen (limited screen). Homozygous familial hypercholesterolemia included findings of a single pathogenic or likely pathogenic (P/LP) variant in LDLR, APOB, or PCSK9. Homozygous familial hypercholesterolemia included 2 (homozygous or compound-heterozygous) P/LP variants in LDLR, APOB, or PCSK9. Autosomal recessive hypercholesterolemia included findings of 2 P/LP variants in LDLRAP1. Autosomal recessive hypercholesterolemia carrier included a single P/LP variant in LDLRAP1. Analyses included only gain-of-function variants in APOB and assumed that the limited-variant screen does not report zygosity.
ity of these tests, then the absolute number of individuals receiving positive findings for FH from limited-variant screening may exceed those identified via comprehensive clinical testing. This could have substantial public health benefits. However, the challenge of negative findings remains, as the lack of genetics-informed clinician involvement with direct-to-consumer approaches may lead to misinterpretation of a negative result as a complete absence of risk for FH.

Although identification of a P/LP variant is part of most diagnostic criteria for FH, diagnosis has traditionally been based only on clinical criteria, including elevated LDL cholesterol levels and family history of premature cardiovascular disease.
However, family history may be inconclusive or unavailable and thus insensitive, and clinical observations such as cholesterol levels do not always meet diagnostic thresholds in some families with FH. Genetic testing is sufficient for diagnosis within clinical diagnostic criteria and can identify affected family members before symptom onset, enabling children to benefit from routine evaluation or treatment at a young age. A 20-year follow-up study of statin therapy in children found that none of those receiving treatment had died of cardiovascular disease before age 40 years, compared with 7% of their parents, who had initiated treatment later in life.9

Unfortunately, genetic testing for FH is underused, with 90% of affected individuals worldwide remaining undiagnosed and only 3.9% of patients with FH in the US having a record of genetic testing.11,25,26 Recent expert statements recommend that patients suspected of having FH be offered genetic testing and that first-degree relatives of genetically positive individuals be screened for FH by lipid profile or genetic testing.11,27,28 In addition, the US Centers for Disease Control and Prevention recommends cascade screening of relatives of individuals with FH.29 While the sequencing data in this study was sourced from a single clinical laboratory, any testing facility that provides high-coverage NGS for FH genes could address the genetic testing components of these recommendations.

Clinicians and consumers need to be aware of the limitations of any genetic test. In clinical settings, where clinicians may use limited-variant screens or comprehensive NGS tests, prior studies show that P/LP variants are identified in only 30% to 70% of individuals with a definite diagnosis of FH by LDL cholesterol levels or other clinical findings per Dutch Lipid Clinical Network scoring or Simon Broome Diagnostic Criteria for FH.24,30,31 Thus, an individual with clinical findings of FH and a negative result from any genetic test may have unidentified variants, and immediate family members remain at up to 50% risk. Further, the potential for false reinsurance or incomplete information after limited-variant screening is not limited to FH. For any genetic condition with allelic heterogeneity in which multiple alleles at the same genetic locus can cause the same disease, analyzing only a limited selection of variants will likely lead to missed or incomplete diagnoses. Future work to pool comprehensive testing results from multiple testing facilities for comparison to limited-variant screening findings is needed to fully understand the disparate outcomes of various genetic technologies.

Although most direct-to-consumer limited-variant screens are used outside of medical care, some users have reported that personal health and health-focused lifestyle changes are motivations for undergoing testing.32-34 The US Food and Drug Administration has stated that “not all direct-to-consumer genetic test companies perform the same set of variants, and therefore may provide different results for the same disease or condition.”35 Moreover, false positives have been reported for 40% of variants detected by third-party companies analyzing raw data obtained from direct-to-consumer genetic testing laboratories.36 Thus, clinical confirmation of any direct-to-consumer testing result is advised to avoid inappropriate changes in care. The US Food and Drug Administration recommends that individuals with positive direct-to-consumer testing results consult with their clinicians about next steps, which should include confirmatory clinical genetic testing.35

Limitations
Limitations of this study include an inability to confirm the molecular diagnoses of FH with clinical diagnoses due to the lack of medical and family histories. Although previous studies have demonstrated a 30% to 70% yield from definite FH cases,24,30,31 the yield in the indication cohort in this study was 27.0%. This should not reflect a diminished sensitivity of the NGS test because the prevalence of FH disease-associated variants in the proactive cohort was similar to frequencies found by other methods. Instead, we suspect that some of the cases in the indication cohort may have had a low prior clinical probability of a positive test result. In addition, our assessment of limited-variant screening was a projection, as we did not have access to data from individuals who have used the comparison assay. Finally, the generalizability of our findings may be hampered by the limited representation of Hispanic and Black/African American individuals in our cohorts. This issue deserves further investigation to identify the cause or causes, which may include care, access, and coverage disparities; economic inequities (especially for the proactive cohort for which testing is largely paid out of pocket); or other reasons.37-39

Conclusions
Overall, our findings demonstrate a potential for significantly reduced clinical sensitivity and false reinsurance among most individuals (61.8% to 68.9%) with disease-causing FH variants when limited-variant, array-based testing is used, especially among Black/African American and Hispanic individuals. Whether testing is obtained directly by a consumer or through a clinical setting, those tested should consult with a genetic counselor or other qualified health care professional to fully understand the benefits and limitations of the different types of genetic testing for FH.
and Drs Truty, Callis, Esplin, Haverfield, Rojahn, and Vatta hold stock from Invitae as employees during the conduct of the study. Dr Garcia reports stocks from Invitae as an employee and stocks from 10x Genomics, Personalis, and 23andMe from previous employment outside the submitted work. Dr Nussbaum reports stocks from Invitae as an employee during the conduct of the study; personal fees from Pfizer, Genome Medical, and Maze Therapeutics outside the submitted work; and is a stockholder in Genome Medical and Maze Therapeutics. Dr Rader reports personal fees from Alnylam Pharmaceuticals, Novartis, Pfizer, and Verve as a scientific advisory board member; is chief scientific advisor for Familial Hypercholesterolemia Foundation; and is a cofounder of Vascular Strategies and Staten Biotechnology during the conduct of the study. No other disclosures were reported.

REFERENCES


Not all genetic testing is the same, and interpretation of genetic testing gains accuracy when a patient’s ancestry is considered. In this issue of JAMA Cardiology, Sturm and colleagues1 explore these issues for familial hypercholesterolemia (FH) genetic testing.

The value of genetic testing for FH has been questioned because severe hypercholesterolemia (ie, low-density lipoprotein cholesterol [LDL-C] >190 mg/dL; to convert to millimoles per liter, multiply by 0.0259), regardless of causative mechanism, merits LDL-C lowering with statins. The availability of costly nonstatin medications has bolstered genetic testing, since results can inform the need for these medications. Furthermore, FH mutations associate with increased coronary artery disease risk even among individuals matched for LDL-C concentrations.2 Classic clinical criteria for FH have become less sensitive, given trends in statin use and saturated fat intake and imprecise family histories. A recent expert panel recommended offering FH genetic testing to individuals of any age in whom FH is clinically strongly suspected.3

Sturm et al1 evaluated 2 distinct types of FH genetic testing by analyzing sequencing data from Invitae, a commercial clinical genetics company. Familial hypercholesterolemia variant status was assigned based on the presence of pathogenic or likely pathogenic variants in LDLR, APOB, PCSK9, or LDLRAP1, using full scanning of the genes’ coding regions using next-generation sequencing (NGS). They compared comprehensive NGS with assessing genotypes for 24 known pathogenic, specific FH variants. This second method mimics a typical analysis taken by direct-to-consumer tests, which rely on array-based variant detection rather than NGS. Genotyping arrays interrogate prespecified variants at reduced cost, whereas NGS detects variation of each base pair and compares findings with a reference human genome.

Among those clinically referred for FH genetic testing, 27.0% had a positive test result using NGS, whereas only 8.4% were identified using the 24-variant approach. Striking differences in yield by race/ethnicity were observed; the 24-variant approach missed 85% and 94% of FH mutations in Hispanic individuals and African American individuals, respectively. Genetic databases overrepresent European ancestry populations and therefore make interpretation of genetic variation more accurate in these cohorts. However, even 64% of the FH mutations in European American individuals would have been missed by the 24-variant.

A negative FH test result from these direct-to-consumer array platforms is limited by reduced sensitivity to detect rare variants and poor curation of recurrent non-European mendelian variants. When FH is strongly clinically suspected, even if array-based FH reporting has negative results, a clinical genetic test should still be considered.