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Phenotypic diagnostic testing for FH

A comparative analysis of phenotypic predictors of mutations in familial hypercholesterolemia

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Context: The gold standard for diagnosing familial hypercholesterolemia (FH) is identification of a causative pathogenic mutation. However, genetic testing is expensive and not widely available.
Objective To compare the validity of the Dutch Lipid Clinic Network (DLCN), Simon Broome (SB), Make Early Diagnosis to Prevent Early Deaths (MEDPED) and American Heart Association (AHA) criteria in predicting an FH-causing mutation.
Design, Setting, and Patients: An adult cohort of unrelated patients referred to a lipid clinic for genetic testing.
Main Outcome Measures: Odds ratio (OR), area-under-the-curve (AUC), sensitivity and specificity.
Results: A pathogenic FH-causing mutation was detected in 30% of 885 patients tested. Elevated LDL-cholesterol and personal or family history of tendon xanthomata were independent predictors of a mutation (ORs range 5.32-15.2, $P<0.001$). Prediction of a mutation for the DLCN and SB definite and MEDPED criteria (ORs 9.4, 11.7. 10.5, respectively) was higher than with the AHA criteria (OR 4.67). The balance of sensitivity and specificity was in decreasing order DLCN definite (Youden Index 0.487), MEDPED (0.457), SB definite (0.274) and AHA criteria (0.253), AUC being significantly higher with DLCN definite and MEDPED than other criteria ($P<0.05$). Pre-treatment LDL-cholesterol and tendon xanthomata had the highest AUC in predicting a mutation.
Conclusions: The DLCN, SB and MEDPED criteria are valid predictors of an FH-causing mutation in patients referred to a lipid clinic, but concordance between these phenotypic criteria is only moderate. Use of pre-treatment LDL-cholesterol and tendon xanthomata alone may be particularly useful for deciding who should be genetically tested for FH.

We compared the validity of four diagnostic tools in predicting an FH-causing mutation. The DLCN, SB definite and MEDPED phenotypes are valid predictors of a mutation in patients with suspected FH.

Introduction

Familial hypercholesterolemia (FH) is a co-dominantly inherited disorder resulting in elevated low density lipoprotein (LDL)-cholesterol concentration and increased risk of premature atherosclerotic cardiovascular disease (ASCVD) (1). FH is primarily caused by mutations in the LDL receptor (*LDLR*), and less commonly by mutations in apolipoprotein B (*APOB*) and proprotein convertase subtilisin/kexin type 9 (*PCSK9*) genes (2). The prevalence of heterozygous FH is estimated to be 1 in 250 in unselected community populations (3-5). While genetic testing is the reference standard for diagnosing FH and has great value in risk assessment and cascade screening, it is expensive and not widely available (6). Deciding

whether to undertake a genetic test in patients with phenotypic FH referred to a clinic is a key consideration.

The clinical diagnosis of FH is based on identifying a personal and family history of premature coronary artery disease (CAD), tendon xanthomata, corneal arcus and elevated LDL-cholesterol (7-9). However, there is no standardised phenotypic diagnosis of FH (10). The most commonly recommended diagnostic tools include the Dutch Lipid Clinic Network (DLCN), the Simon Broome (SB) and Make Early Diagnosis to Prevent Early Deaths (MEDPED) criteria (7-9). However, a detailed family history and physical stigmata required by the DLCN and SB criteria may be difficult to elicit. A simplified diagnosis of FH, based on LDL-cholesterol level and family history of elevated LDL-cholesterol or premature CAD, was recently proposed by the American Heart Association (AHA) (11).

We previously reported on the characteristics of patients with FH in a service for lipid disorders in Western Australia (6, 12). We now compare the diagnostic validity of the aforementioned clinical tools in discriminating between the presence and absence of a pathogenic gene variant causative of FH. We thereby aimed to identify the optimal phenotype for predicting a mutation and how best to utilize genetic testing for FH in the clinic.

Materials and Methods

Study design

A cross-sectional comparison was undertaken of unrelated patients aged ≥18 years with or without a recognised mutation causative of FH. The care of these patients has been previously described (6, 12). In brief, adult patients with suspected FH were recruited via referral from general practice, coronary care or other specialists. Reasons for suspecting FH were generally an elevated plasma LDL-cholesterol with a family or personal history of CAD. Patients were evaluated by specialist physicians at the Lipid Disorders Clinic at Royal Perth Hospital (RPH, Perth, Western Australia); if the diagnosis was at least possible FH, they were genetically tested after appropriate counselling and consent. The study was approved by the RPH Human Research Ethics Committee.

Clinical data

Age, gender, history of coronary and other vascular disease, diabetes, hypertension, smoking status, examination findings (weight, height, blood pressure), and medications were recorded. The definition of CAD, diabetes, hypertension and obesity were previously described (6, 12).

Phenotypic diagnosis of FH

Details were obtained of family and personal history of hypercholesterolemia, premature coronary or vascular disease, tendon xanthomata (bilateral, subcutaneous nodules on Achilles tendons or at ligamentous insertions) and corneal arcus; pre-treatment plasma LDL-cholesterol concentration was recorded. Where a pre-treatment plasma lipid profile was not available (25% of the cohort), a correction that accounted for type, dose and frequency of statins and use of ezetimibe (Supplementary Table 1), was used to estimate pre-treatment LDL-cholesterol concentrations (13). Patients were classified phenotypically, using the same individual variable, according to the DLCN, SB, MEDPED and AHA diagnostic criteria (Supplementary Table 2) (7-11). Since approximately 30% of Lp(a) mass is cholesterol (14), in subsidiary analyses LDL-cholesterol was also adjusted for the cholesterol content of Lp(a) by subtracting 30% of total Lp(a) mass from the measured LDL-cholesterol concentration.

Genetic diagnosis of FH

The diagnosis of FH was based on the presence of a pathogenic gene variant. DNA extraction, multiplex ligation-dependent probe amplification (MLPA) and Sanger sequencing of the 18 exons of the *LDLR*, exon 7 of *PCSK9*, and part of *APOB* exons 26 and 29 was performed as previously described (6). In 10% of patients, genetic testing was based on next-

generation sequencing (NGS), performed by Ion Torrent sequencing using a TargetSeq (Life Technologies) custom capture panel of lipid genes and polymorphisms, derived from LipidSeq (15). Mutation detection rates with NGS (29%) and Sanger sequencing (30%) were not significantly different. MLPA was performed in all patients with DLCN probable or definite FH in whom Sanger sequencing or NGS did not identify a mutation.

Laboratory analyses

Venous blood samples were collected during clinic visits. Plasma cholesterol, HDL-cholesterol and triglyceride concentrations were determined by standard enzymatic methods. LDL-cholesterol was calculated by the Friedewald equation (16), but in patients with plasma triglyceride >4.5mmol/L a direct LDL-cholesterol assay was employed. Total apoB and Lp(a) (Quantia Lp(a) assay and standards) were determined by immunoassay (Abbott Laboratories, Abbott Park, IL), with inter-assay coefficient of variation of <5%.

Statistical analyses

All data were analysed using SPSS (version 21, Chicago, IL). Differences in clinical characteristics between FH index cases with and without a pathogenic mutation (*LDLR*, *APOB* and *PCSK9*) were tested with chi-squared or independent *t*-tests. Skewed variables, including plasma triglyceride and Lp(a) concentrations, were log-transformed. Logistic regression was used to assess prediction of a mutation. The discriminant value of plasma LDL-cholesterol concentration and the DLCN score in predicting a mutation were depicted using receiver operator characteristic (ROC) area-under-the-curve (AUC). Cohen's kappa coefficient was used to measure the phenotypic agreement between DLCN, SB, MEDPED and AHA criteria. The sensitivity, specificity, positive predictive valve (PPV), negative predictive value (NPV), and Youden index were employed to compare the diagnostic value of the clinical tools and their components in predicting a mutation. Improvement in prediction of a mutation with individual variable relative to the DLCN and SB definitions was estimated as differences between the AUCs and compared using C-statistics. Statistical significance was defined at the 5% level.

RESULTS

A total of 931 adult patients with at least “possible” FH according to the DLCN criterion (score ≥3) were genetically screened for a causative mutation for FH from March 2007 to September 2017. Patients with homozygous FH, compound or double heterozygous FH (n=6), *LDLR* or *APOB* variants of uncertain significance (n=30), pathogenic or uncertain *ABCG5/8* variants (n=5), or those with frank secondary causes of hypercholesterolemia (n=5) were excluded from the analysis. Of the remaining 885 index cases, 267 (30%) of the screened cases had a pathogenic FH-causing mutation: 237 were found to have mutation in the *LDLR* gene (60% missense, 20% nonsense/frameshift, 11% splice, 8% large deletion/duplication and 1% promoter variants), while others were heterozygous for *APOB* (n=25), *PCSK9* (n=3) and *APOE* p.Leu167del (n=2) mutations.

The characteristics of the 885 patients with (M+) and without (M-) a mutation are shown in Table 1. M+ individuals were older, and had a higher frequency of non-Caucasian origin, tendon xanthomata and corneal arcus (P<0.01 for all) and hypertension (P<0.05) than M- patients. There were no significant differences in the proportion of men, smokers, or patients with CAD or cerebral/peripheral vascular disease, type 2 diabetes or obesity, and use of anti-hypertensive agents or aspirin between the M+ and M- groups. Compared with M- individuals, M+ individuals had higher total cholesterol, non-HDL-cholesterol, LDL-cholesterol (treated and pre-treatment) and apoB levels, and more frequent use of lipid-lowering medication than M- individuals (P<0.01 for all). Plasma HDL-cholesterol and Lp(a) levels were not significantly different between the two groups (P>0.05 for both), but plasma

triglyceride levels were significantly higher in the M- group. M+ individuals had a higher DLCN score (10.6±4.0 vs 6.2±2.5) and prevalence of DLCN definite FH (65% vs 17%), SB definite FH (31% vs 4%), MEDPED defined FH (79% vs 26%) and AHA defined FH (89% vs 64%) than M- individuals (P<0.001 for all).

In univariate regression analysis (Table 2), family history of ASCVD (premature CAD and/or cerebral/peripheral vascular disease) or elevated LDL-cholesterol, presence of tendon xanthomata, corneal arcus and elevated LDL-cholesterol were all significant predictors of a mutation (odds ratios [ORs] range 3.00-27.0, P<0.001 for all). Using the DLCN criteria, the ORs for an FH-causing mutation were 9.47 (95%CI 6.81-13.2) and 8.58 (5.40-13.6) for definite and for probable/definite FH, respectively. The ORs for SB definite, possible/definite FH, MEDPED and AHA criteria were 11.7 (7.15-19.1), 7.79 (4.57-13.3), 10.5 (7.48-14.9) and 4.67 (3.07-7.09), respectively. Age and ethnicity were also significant predictors of a mutation (OR 0.977, 95%CI 0.966-0.987 and 2.02, 95%CI 1.32-3.09, respectively); i.e. mutations were more likely in younger and non-Caucasian patients. As seen in Table 2, family history, physical stigmata and elevated LDL-cholesterol remained independent predictors of the presence of a mutation in multivariate regression models. In stepwise regression analysis including tendon xanthomata, family history of physical stigmata and the DLCN definite criteria, elevated LDL-cholesterol (≥8.5mmol/L) was the best predictor of the presence of a mutation (β-coefficient 2.22, SE 0.351). The β-coefficients for tendon xanthomata, family history of physical stigmata and DLCN definite criteria were 0.924 (SE 0.319), 2.01 (0.278) and 1.01 (0.235), respectively. After adjusting for Lp(a)-cholesterol, elevated LDL-cholesterol remained an independent predictor of the presence of a mutation in the multivariate regression models. Inclusion of age, gender and ethnicity as independent variables also did not alter the significance of the aforementioned variables in detecting a mutation.

Table 3 shows the concordance and discordance rates between the four clinical diagnostic tools. There was a "moderate" agreement between DLCN and SB definite ($\varkappa$=0.455), with an overall discordance rate of 19%. However, there was a "poor" agreement between DLCN probable/definite and MEDPED criteria ($\varkappa$=0.120, concordance rate 67%, discordance rate 33%), between SB possible/definite and MEDPED criteria ($\varkappa$=0.185, concordance rate 56%, discordance rate 44%), and between the AHA criteria and DLCN definite ($\varkappa$=0.120, concordance rate 49%, discordance rate 51%), SB definite FH ($\varkappa$=0.028, concordance rate 36%, discordance rate 64%) and MEDPED criteria ($\varkappa$=0.176, concordance rate 56%, discordance rate 44%). The kappa coefficient between the DLCN probable/definite and the SB possible/definite and AHA criteria was 0.378 and 0.338, respectively, and between SB definite and MEDPED criteria 0.205, reflecting "fair" agreement between them. There was a "very good" agreement between the SB possible/definite and AHA criteria ($\varkappa$=0.908, concordance rate 99%, discordance rate 1%).

The ROC curve for LDL-cholesterol and DLCN score in predicting an FH-causing mutation was shown in Supplementary Figure 1. The higher the LDL-cholesterol levels and DLCN score, the greater the specificity and the lower the sensitivity in detecting a mutation. The ROC curve for LDL-cholesterol (AUC 0.835; 95%CI 0.806-0.865) and for DLCN score (AUC 0.816; 95%CI 0.784-0.847) were comparable in detecting a mutation (difference between ROC areas 0.020; 95%CI -0.001-0.048; P=0.164). The AUC for LDL-cholesterol, after adjusting for Lp(a)-cholesterol, in detecting a mutation was 0.841 (95%CI 0.812-0.870) which was also not significantly different from that for DLCN score as a continuous variable (difference between ROC areas 0.011; 95%CI -0.017-0.040; P=0.453).

Table 4 shows the sensitivity, specificity, PPV, NPV, Youden index and AUC for different diagnostic criteria in detecting an FH-causing mutation. A family history of physical stigmata, the presence of tendon xanthomata and an LDL-cholesterol ≥8.5 mmol/L had high

specificities (>95% for all), but relatively low sensitivities (range 31.1-36.7%) in detecting a mutation. A family history of premature CAD or hypercholesterolemia had high sensitivity (90.2%), but low specificity (24.4%) in detecting a mutation. Lower plasma LDL-cholesterol concentrations were more sensitive (from 36.7% to 98.9%) but less specific (from 97.9% to 14.7%) in detecting a mutation. The highest Youden index among the individual diagnostic components was 0.485 with an LDL-cholesterol ≥6.5mmol/L.

With the composite diagnostic criteria, Table 4 also shows that SB definite FH had a higher specificity (96.3%) but lower sensitivity (31.1%) in detecting a mutation than DLCN definite FH (specificity 83.5%, sensitivity 65.2%) and MEDPED criteria (specificity 88.9%, sensitivity 56.8%). However, the DLCN definite FH (Youden index 0.487) and MEDPED criteria (Youden index 0.457) had a better balance between sensitivity and specificity than the SB definite (Youden index 0.274) in detecting a mutation. While the AHA criteria had a high sensitivity (89.1%), its specificity (36.2%) was lower compared with DLCN definite, SB Definite or MEDPED criteria. The Youden indices for the composite were in decreasing order: DLCN definite, MEDPED criteria, DLCN probable/definite, SB definite, SB possible/definite and AHA criteria.

With reference to a DLCN definite diagnosis, the AUCs were significantly less (P<0.05 for all) with family history of ASCVD (or elevated LDL-cholesterol), family and personal history of physical stigmata, personal history of ASCVD, elevated LDL-cholesterol (≥5.0mmol/L or ≥8.5mmol/L), DLCN probable/definite FH, SB possible/definite FH and AHA criteria (AUCs range -0.067 to -0.239). With reference to a SB definite diagnosis, the AUCs were significantly less (P<0.05 for all) with family history of ASCVD (or elevated LDL-cholesterol), personal history of ASCVD, corneal arcus and elevated LDL-cholesterol ≥5.0 mmol/L (AUCs range -0.069 to -0.133). The AUC for an LDL-cholesterol ≥6.5 mmol/L was significantly higher than a SB definite diagnosis (0.106, 95%CI 0.067-0.144) but not different from a DLCN definite diagnosis (-0.001, 95%CI -0.037-0.035). With reference to a DLCN probable/definite or SB possible/definite diagnosis, the AUCs were significantly less with family history of ASCVD (or elevated LDL-cholesterol), personal history of ASCVD and corneal arcus and LDL-cholesterol ≥5.0 mmol/L (AUCs range -0.062 to -0.173). The AUC for the MEDPED criteria was similar to the DLCN definite FH (0.487 vs 0.457, P>0.05), but significantly higher compared with the SB definite, DLCN probable/definite or SB possible/definite diagnosis (P<0.05 for all). While family history of physical stigmata showed no significant difference in AUCs, an LDL-cholesterol ≥6.5mmol/L had a significant higher AUCs when compared with the DLCN probable/definite or SB possible/definite diagnosis (0.066 and 0.107, respectively, P<0.001).

Discussion

In a large sample of patients referred to a specialist clinic, we demonstrated that the DLCN, SB and MEDPED phenotypes were valid predictors of an FH-causing mutation, the AHA criteria having the lowest diagnostic specificity. Among individual phenotypic components, the pre-treatment LDL-cholesterol and presence of tendon xanthomata afforded the best discriminant value in predicting a mutation that overall matched the DLCN definite, SB definite and MEDPED criteria. The concordance between these phenotypic tools for FH was, however, only moderate.

Several studies have examined the relationship between clinical diagnostic tools and genetically defined FH. Damgaard et al reported that the DLCN and SB criteria were comparably effective in identifying most carriers of an FH-causing mutation, with specificities >85%, but sensitivities of <45% (17); the discriminant value of individual diagnostic components was not investigated. The diagnostic yield of the DLCN and SB criteria in predicting the common FH-causing mutations was later confirmed in a larger

community population, but there was limited information on family history and no records of physical stigmata of FH (4). Haralambos et al reported on the value of a modified DLCN criteria in predicting FH-causing mutations in Wales, but the findings were limited by lack of genetic data in individuals with lower DLCN score (13). Silva et al found that the DLCN score was a significant predictor of a mutation, but SB diagnostic criteria were not examined (18). Civeira et al also reported that the MEDPED criteria had high sensitivity and specificity in predicting a mutation in Spanish patients, but tendon xanthomata and age-adjusted LDL-cholesterol were the most significant predictors of a mutation (19). We extend previous reports by comparing the discriminant value of different phenotypic criteria in predicting an FH-causing mutation among referrals to a specialist clinic.

That a higher proportion of M+ than M- patients in our study fulfilled a phenotypic diagnosis of FH according to the DLCN definite, SB definite, MEDPED and AHA criteria was anticipated. Tendon xanthomata and markedly elevated LDL-cholesterol are classical hallmarks of FH (7-9, 11). Consistent with previous studies (18, 19, 21), the presence of tendon xanthomata and/or corneal arcus in the potential index cases and family members were independent predictors of the presence of an FH-causing mutation (Table 2). Likewise, a pre-treatment LDL-cholesterol above 4.9 mmol/L was confirmed as an independent predictor of a mutation. Given that tendon xanthomata and elevated LDL-cholesterol are major criteria for the DLCN and SB diagnostic tools (7, 9), it was anticipated that these phenotypic components were highly predictive of a mutation. While the AHA tool was also predictive of a mutation, its specificity and PPV were significantly less than DLCN and SB definite and MEDPED criteria. In agreement with Civeira et al (19), we found that a history of premature CAD was not a significant predictor of a mutation. This accords with recent understanding that the major causes of premature CAD are due to multigenic and adverse lifestyle factors. As suggested elsewhere, familial combined hyperlipidemia and common hypercholesterolemia with elevated Lp(a) are frequent phenocopies of FH (22).

Although we studied referrals to a specialist clinic, approximately 30% of patients with a definite phenotypic FH by DLCN and SB criteria did not have an FH-causing mutation. This points to polygenic hypercholesterolemia (attributed to common, small-effect LDL-cholesterol raising alleles), or unrecognized genetic causes of autosomal dominant hypercholesterolemia (23, 24). Conversely, more than 15% of patients with a mutation were not identified by a DLCN or SB definite diagnosis. This suggests less severe FH phenotypes associated with pathogenic *LDLR* variants causing less LDL-receptor dysfunction, reduced genetic penetrance and/or to co-inheritance of protective genes (25). Earlier detection and treatment with statins can also modify phenotypic FH (26). The phenotypic diagnosis of FH appears more dependent on the pre-treatment LDL-cholesterol than on other clinical features of FH, consistent with the earlier MEDPED recommendations (8), although clinical stigmata allow greater refinement of the diagnosis (8, 27).

The strength of the study was the use of well standardised, comprehensive criteria for characterising patients. However, there were limitations. The sample was predominantly *Caucasian, but reflected the ethnic distribution of the Australian population.* That the likelihood of an FH-causing mutation was increased in non-Caucasians reflects higher specificity in this group of applying Caucasian criteria. The diagnosis of FH in children based on LDL-cholesterol were not examined, but are reviewed elsewhere (28). Because of ascertainment bias, our results may have limited applicability to the community diagnosis of FH, but our conclusions may be valuable when applied to targeted screening of electronic health records for FH in primary care (29). Approximately 25% of our patients were on cholesterol-lowering medication and pre-treatment LDL-cholesterol was derived using adjustments that might not have fully reflected true values (13). Testing for cholesterol allelic

gene scores might have helped identify patients with isolated polygenic hypercholesterolemia (24).

Implications

The reference diagnosis of FH is the identification of a pathogenic mutation that affects the clearance of LDL via the LDL-receptor pathway (1, 2). Genetic testing may be used to predict ASCVD risk (19), but is currently expensive and not widely available. Moreover, not all patients consent to genetic testing owing to personal preferences and potential discrimination by employers and insurers (3, 5, 11, 25). Knowledge of the phenotypic predictors of a mutation may be useful for improving the accuracy of diagnosis where genetic testing is not available, or not consented to, and for rationing genetic testing where resources are limited.

With rising awareness of FH in primary care, an increasing number of patients will be referred to specialist clinics for confirmation of the diagnosis. Because of limited resources, it may be necessary to phenotypically identify patients who are very likely to have FH in whom genetic confirmation may be less valuable (unless combined with cascade screening), patients with an intermediate clinical likelihood in whom genetic testing may be particularly useful, and patients with low likelihood in whom genetic testing could be wasteful. Cascade screening for FH using LDL-cholesterol and genetic testing is potentially cost-effective (27, 30, 31). Phenotypic cascade testing from index cases with a definite clinical diagnosis is also intuitively more cost-effective than from index cases with lesser probabilities of FH. Hence, genetic testing of index cases with an intermediate probability of FH may offer an effective use of laboratory resources in the context of cascade testing.

Based on our results, Figure 1 shows the application of the four phenotypic diagnostic tools to the detection of a mutation and to enabling decision regarding genetic testing in patients referred to clinics. In this scheme, genetic testing could be recommended in patients with an intermediate probability of having a mutation and could be considered in patients with a low phenotypic probability in whom the clinical suspicion of FH is high. A caveat is that although prediction of a mutation appears statistically comparable among DLCN, SB and MEDPED criteria, the phenotypic classification of patients can be discordant (Table 3).

Given that information on individual family members are not always available, a simpler scheme shown in Figure 2 utilizes the pre-treatment LDL-cholesterol and presence of tendon xanthomata in potential index cases to predict the likelihood of a mutation and value of genetic testing. Use of pre-treatment LDL-cholesterol as the primary criterion accords with earlier MEDPED recommendations (8) and is useful for identifying patients with intermediate likelihood of FH in whom genetic confirmation is required. Inclusion of tendon xanthomata as a second criterion further improves the specificity of the diagnosis of FH.

In adult patients referred to a specialist lipid clinic, prediction of an FH-causing mutation, or the decision to request a genetic test, could simply be based on the pre-treatment LDL-cholesterol and presence of a personal (or family) history of tendon xanthomata. While florid tendon xanthomata are currently less frequent among heterozygous FH (32), their earlier detection may be enhanced with ultrasonography (33). This may provide a more standardised approach to the diagnosis of FH and overcome the notable discordance between DLCN and SB clinical diagnostic criteria. Country-specific LDL-cholesterol levels should replace the original MEDPED criteria (8, 27). Services with requisite laboratory and clinical resources may elect to genetically test all patients referred with a putative diagnosis of FH, but the cost-effectiveness of this policy remains to be evaluated.

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Figure 1 Flowcharts showing the use of the phenotypic (A) Dutch Lipid Clinic Network, (B) Simon Broom, (C) Make Early Diagnosis to Prevent Early Deaths and (D) American Heart Association diagnostic tools in predicting the likelihood of a FH-causing mutation and assisting decisions on whether to undertake genetic testing. Genetic testing is arbitrarily not routinely recommended where probability of a mutation is <15%, is recommended when the probability of a mutation is 15-60% and is optional when the probability of a mutation >60%.

Figure 2 Flowchart showing use of LDL-cholesterol concentration and presence of tendon xanthomata in predicting the likelihood of a FH-causing mutation and assisting decisions on whether to undertake genetic testing. Genetic testing is arbitrarily not routinely recommended where probability of a mutation is <15%, is recommended when the probability of a mutation is 15-60% and is optional when the probability of a mutation >60%.

Table 1. Demographic, clinical and biochemical characteristics of the patients with FH in relation to presence (Mutation +) and absence (Mutation -) of an FH-causing mutation

Table 1. Demographic, clinical and biochemical characteristics of the patients with FH in relation to presence (Mutation +) and absence (Mutation -) of an FH-causing mutation

	Total	Mutation +	Mutation	*P*
Number of subjects	885	267	618	
Age at diagnosis (years)	51 ± 13	48 ± 14	52 ± 13	<0.001
Male (%)	46	41	47	0.091
Ethnicity Caucasian (%)	89	84	91	0.004
Asian (%)	6	10	5	
Other (Mixed/Aboriginal/African/Middle Eastern) (%)	5	6	4	
Early CAD (%)	44	48	43	0.141
Early cerebral or peripheral vascular disease (%)	4	5	4	0.719
Tendon xanthomata (%)	12	31	4	<0.001
Corneal arcus (%)	27	36	24	0.001
Smoking (ever) (%)	42	41	42	0.767
Hypertension (%)	29	23	32	0.012
Type 2 Diabetes (%)	7	5	7	0.375
Obesity (BMI >30 kg/m^2) (%)	30	28	32	0.386
On lipid-lowering medication (%)	66	75	63	0.001
On anti-hypertensive medication (%)	29	27	30	0.422
On aspirin (%)	28	31	27	0.223
Total cholesterol (mmol/L)	5.7 ± 1.8	6.0 ± 2.0	5.6 ± 1.7	0.018
Triglyceride (mmol/L)	1.8 ± 1.1	1.4 ± 0.7	1.9 ± 1.2	<0.001
HDL-cholesterol (mmol/L)	1.4 ± 0.6	1.3 ± 0.4	1.4 ± 0.6	0.168
Non-HDL-cholesterol (mmol/L)	4.4 ± 1.8	4.6 ± 2.0	4.3 ± 1.6	0.007
LDL-cholesterol (mmol/L)	3.6 ± 1.6	3.9 ± 1.9	3.4 ± 1.5	<0.001
Pre-treated LDL-cholesterol (mmol/L)	6.6 ± 1.7	8.1 ± 1.8	6.0 ± 1.1	<0.001
Lipoprotein(a) (g/L)	0.30 (0.28-0.32)	0.27 (0.24-0.31	0.31 (0.29-0.35)	0.073
ApoB (g/L)	1.1 ± 0.4	1.2 ± 0.4	1.1 ± 0.3	0.001
Dutch Lipid Clinic Network (DLCN) criteria score**	7.6 ± 3.6	10.6 ± 4.0	6.2 ± 2.5	<0.001
DLCN category*** Definite FH (%)	31	65	17	<0.001
Probable FH (%)	36	27	40	<0.001
Possible FH (%)	33	8	43	<0.001
Simon Broome*** Definite FH (%)	12	31	4	<0.001
Possible FH (%)	63	63	63	0.685
MEDPED Probable Heterozygous FH (%)	42	79	26	<0.001
American Heart Association Heterozygous FH (%)	71	89	64	<0.001

Values are expressed as mean ± SD or geometric mean (95%CI); *P*-value for Mutation + vs Mutation -; **DLCN score refers to the score without the genetic analysis component [ie. family history, clinical history, physical examination (tendon xanthomata and corneal arcus) and LDL-cholesterol level only]; tendon xanthomata were diagnosed with reference to the Achilles tendons; ***DLCN and Simon Broome FH categories do not take account of the genetic analysis component into the classification.

Table 2. Predictors of the presence of an FH causing mutation according to individual phenotypic criteria and composite diagnostic tools for FH

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	Univariate logistic regression			Multivariate logistic regression		
	OR	95% CI	*P*	OR†	95%CI	*P*
Individual phenotypic criteria						
Family history						
Premature CAD and/or vascular disease or elevated LDL-cholesterol	3.00	1.92-4.67	<0.001	3.24	1.86-5.64	<0.001
Tendinous xanthomata and/or corneal arcus	12.0	7.39-19.4	<0.001	8.65	4.98-15.03	<0.001
Clinical history						
Presence of premature CAD	1.24	0.93-1.66	0.139			
Presence of premature cerebral or PVD	0.82	0.39-1.71	0.597			
Physical examination						
Tendinous xanthomata	10.7	6.64-17.2	<0.001	5.32	2.94-9.61	<0.001
Corneal arcus	1.75	1.29-2.40	<0.001	1.55	1.04-2.31	0.033
Pre-treated LDL-cholesterol (mmol/L)						
LDL-cholesterol *	2.81	2.41-3.27	<0.001			
LDL-cholesterol ≥8.5	27.0	14.8-49.3	<0.001	16.1	8.42-30.6	<0.001

LDL-cholesterol ≥6.5	8.77	6.20-12.4	<0.001	7.54	5.11-11.2	<0.001‡
LDL-cholesterol ≥5.0	15.2	4.77-48.5	<0.001	14.9	4.48-49.3	<0.001‡
Composite diagnostic tools						
DLCN score *	1.49	1.41-1.58	<0.001			
DLCN Definite FH**	9.47	6.81-13.2	<0.001			
DLCN Probable/Definite FH**	8.58	5.40-13.6	<0.001			
Simon Broome Definite FH**	11.7	7.15-19.1	<0.001			
Simon Broome Possible/Definite FH	7.79	4.57-13.3	<0.001			
MEDPED Probable Heterozygous FH	10.5	7.48-14.9	<0.001			
American Heart Association Heterozygous FH	4.67	3.07-7.09	<0.001			

*Expressed as a continuous variable; **refers to the classification without the genetic analysis component. †Multivariate logistic regression (variables enter on model: family history of premature CAD and/or vascular disease or elevated LDL-cholesterol, family history of tendon xanthomata and/or corneal arcus, tendon xanthomata, corneal arcus, and pre-treated LDL-cholesterol ≥8.5mmol/L.); ‡Replacing LDL-cholesterol ≥8.5mmol/L with lower LDL-cholesterol cut-off.

Table 3. Concordance and discordance rates for diagnostic outcomes according to Dutch Lipid Criteria Network (DLCN), Simon Broome (SB), Make Early Diagnosis to Prevent Early Deaths (MEDPED) and American Heart Association (AHA) phenotypic tools

Table 3. Concordance and discordance rates for diagnostic outcomes according to Dutch Lipid Criteria Network (DLCN), Simon Broome (SB), Make Early Diagnosis to Prevent Early Deaths (MEDPED) and American Heart Association (AHA) phenotypic tools.

	Concordance rate	Discordance rate	Kappa coefficient*
DLCN definite FH vs SB definite FH			
For total group (n=885)	81%	19%	0.455
For positives (n=276)**	38%	62%	
For negatives (n=609)**	98%	2%	
DLCN probable/definite FH vs SB possible/definite FH			
For total group (n=885)	74%	26%	0.378
For positives (n=594)**	87%	13%	
For negatives (n=291)**	49%	51%	
DLCN definite FH vs MEDPED FH			
For total group (n=885)	73%	27%	0.3717
For positives (n=276)**	73%	27%	
For negatives (n=609)**	72%	28%	
DLCN probable/definite FH vs MEDPED FH			
For total group (n=885)	67%	33%	0.120
For positives (n=276)**	56%	44%	
For negatives (n=609)**	88%	12%	
DLCN definite FH vs AHA FH			
For total group (n=885)	49%	51%	0.120
For positives (n=276)**	83%	17%	
For negatives (n=609)**	34%	66%	
DLCN probable/definite FH vs AHA FH			
For total group (n=885)	71%	29%	0.338
For positives (n=594)**	82%	18%	
For negatives (n=291)**	49%	51%	
SB definite FH vs MEDPED FH			
For total group (n=885)	65%	35%	0.205
For positives (n=106)**	79%	21%	
For negatives (n=779)**	63%	37%	
SB definite FH vs AHA FH			
For total group (n=885)	36%	64%	0.028
For positives (n=106)**	79%	21%	
For negatives (n=779)**	30%	70%	
SB possible/definite FH vs MEDPED FH			
For total group (n=885)	56%	44%	0.185
For positives (n=664)**	48%	52%	
For negatives (n=221)**	78%	22%	
SB possible/definite FH vs AHA FH			
For total group (n=885)	99%	1%	0.908
For positives (n=664)**	95%	5%	
For negatives (n=221)**	100%	0%	

MEDPED FH vs AHA FH			
For total group (n=885)	56%	44%	0.176
For positives (n=370)**	48%	52%	
For negatives (n=515)**	75%	25%	

*Poor agreement < 0.20, fair agreement 0.20 to 0.40, moderate agreement 0.40 to 0.60, good agreement 0.60 to 0.80 and very good agreement 0.80 to 1.00

**Positives and negatives refer to outcomes by the former phenotypic diagnostic category.

Table 4. Sensitivity, specificity, positive (PPV) and negative predictive value (NPV), Youden index and area under curve (AUC) of diagnostic test for FH

Table 4. Sensitivity, specificity, positive (PPV) and negative predictive value (NPV), Youden index and area under curve (AUC) of diagnostic test for FH.

Criteria	Sensitivity % (95% CI)	Specificity % (95% CI])	PPV %, (95% CI)	NPV %, [95% CI])	Youden index	AUC, (95% CI)	Change in AUC, Relative to, DLCN definite FH**, (95% CI)	Change in AUC, Relative to, SB definite, FH**, (95% CI)	Change in AUC, Relative to, DLCN definite/ probable FH**, (95% CI)	Change in AUC, Relative to, SB definite/possible FH**, (95% CI),
Individual diagnostic criteria										
Family history of premature ASCVD or elevated LDL-cholesterol,	90.2 (85.9, 93.4),	24.4 (21.1, 28.1),	34.0 (30.5, 37.7),	85.3 (79.0, 90.0),	0.146,	0.573 (0.534, 0.613),	-0.170* (-0.211, -0.128),	-0.063* (-0.103, -0.024),	-0.103* (-0.136, -0.070),	-0.062* (-0.082, -0.043),
Family History of tendon xanthomata and/or corneal arcus	32.5, (27.1, 38.6)	96.1, (94.2, 97.4)	78.4, (69.4, 85.4)	76.7, (73.5, 79.6)	0.286	0.644, (0.601, 0.686)	-0.100*, (-0.138, -0.061)	0.007, (-0.030, 0.044)	-0.033, (-0.071, 0.005)	0.008, (-0.029, 0.044)
Presence of premature CAD	47.9, (41.8, 54.1)	57.4, (53.4, 61.4)	32.7, (28.2, 37.7)	71.9, (67.6, 75.7)	0.053	0.527, (0.485, 0.568)	-0.216*, (-0.260, -0.172)	-0.110*, (-0.156, -0.064)	-0.150*, (-0.188, -0.111)	-0.109*, (-0.153, -0.065)
Presence of premature cerebral or PVD	3.74, (1.91, 6.99)	95.7, (94.1, 96.9)	26.3, (14.0, 43.4)	69.7, (66.4, 72.7)	0.001	0.496 , (0.455 0.537)	-0.239*, (-0.275, -0.204)	-0.133*, (-0.166, -0.100)	-0.173*, (-0.202, -0.143)	-0.132*, (-0.160, -0.104)
Tendon xanthomata	31.1, (25.7, 37.1)	96.0, (94.0-97.3)	76.9, (67.6, 84.2)	76.3, (73.1, 79.2)	0.271	0.635, (0.592, 0.678)	-0.108*, (-0.140, -0.077)	-0.002, (-0.004, 0.001)	-0.041*, (-0.077, -0.006)	-0.001, (-0.036, 0.034)
Corneal arcus	35.6, (29.9, 41.7)	76.1, (72.4, 79.3)	39.1, (33.0, 45.4)	73.2, (69.6, 76.6)	0.117	0.558, (0.516, 0.600)	-0.185*, (-0.219, -0.151)	-0.079*, (-0.119, -0.038)	-0.118*, (-0.154, -0.083)	-0.078*, (-0.120, -0.036)
LDL-cholesterol ≥8.5 mmol/L	36.7, (31.0, 42.8)	97.9, (96.3, 98.8)	88.3, (80.5, 93.4)	78.1, (75.0, 81.0)	0.346	0.673, (0.631, 0.715)	-0.070*, (-0.101, -0.039)	0.036*, (0.001, -0.071)	-0.003, (-0.039, 0.032)	0.037*, (0.001, 0.073)
LDL-cholesterol ≥6.5 mmol/L	80.5, (75.2, 85.0)	68.0, (64.1, 71.6)	52.1, (47.1, 57.0)	89.0, (85.7, 91.6)	0.485	0.742, (0.707, 0.778)	-0.001, (-0.037, 0.035)	0.106*, (0.067, 0.144)	0.066*, (0.037, 0.095)	0.107*, (0.071, 0.143)
LDL-cholesterol ≥5.0 mmol/L	98.9, (96.5, 99.7)	14.7, (12.1, 17.8)	33.4, (30.1, 36.8)	96.8, (90.3, 99.2)	0.136	0.568, (0.529, 0.607)	-0.175*, (-0.209, -0.141)	-0.069*, (-0.101, -0.037)	-0.108*, (-0.136, -0.081)	-0.068*, (-0.089, -0.046)
Composite diagnostic tools										
DLCN Definite FH,	65.2 (59.1, 70.8),	83.5 (80.3, 86.3),	63.0 (57.0, 68.7),	84.7 (81.6, 87.4),	0.487,	0.743 (0.705, 0.781),	**Reference,**	0.106* (0.075, 0.138),	0.067* (0.034, 0.100),	0.107* (0.070, 0.145),
DLCN Probable/Definite FH	91.8, (87.6, 94.6)	43.5, (39.6, 47.5)	41.2, (37.2, 45.3)	92.4, (88.6, 95.1)	0.353	0.676, (0.641, 0.712)	-0.067*, (-0.100, -0.034)	0.040*, (0.004, 0.075)	**Reference**	0.041*, (0.011, 0.070)
Simon Broome Definite FH	31.1, (25.7, 37.1)	96.3, (94.4, 97.6)	78.3, (69.0, 85.5)	76.4, (73.2, 79.3)	0.274	0.637, (0.594, 0.680)	-0.106*, (-0.138, -0.075)	**Reference**	-0.040*, (-0.075, -0.004)	0.001, (-0.034, 0.036)
Simon Broome Possible/Definite FH	94.0, (90.3, 96.4)	33.2, (29.5, 37.1)	37.8, (34.1, 41.6)	92.8, (88.3, 95.7)	0.272	0.636, (0.599, 0.673)	-0.107*, (-0.145, -0.070)	-0.001, (-0.036, 0.034)	-0.041*, (-0.070, -0.011)	**Reference**

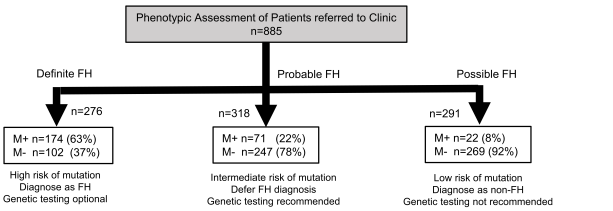
MEDPED Probable FH	56.8, (51.5, 61.8)	88.9, (85.8, 91.4)	78.7, (73.1, 83.3)	74.1, (70.4, 77.5)	0.457	0.764, (0.729, 0.799)	0.021, (-0.009, 0.051)	0.088* (0.058, 0.118)	0.127*, (0.097, -0.157)	0.128*, (0.098, 0.158)
American Heart Association FH	89.1, (84.6, 92.5)	36.2, (32.5, 40.2)	37.7, (33.9, 41.6)	88.5, (83.8, 92.1)	0.253	0.627, (0.589, 0.665)	-0.116*, (-0.158, -0.075)	-0.010, (-0.051, 0.031)	-0.050*, (-0.082, -0.017)	-0.009, (-0.024, 0.006)

**A positive change (delta) in AUC value indicates an increased discriminant value in detecting an FH mutation compared with the reference test

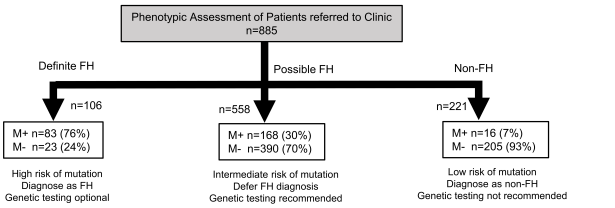
**A negative change (delta) in AUC value indicates a decreased discriminant value in detecting an FH mutation compared with the reference test

*indicates significant differences in delta AUC compared with the reference using C-statistics; $P<0.05$.

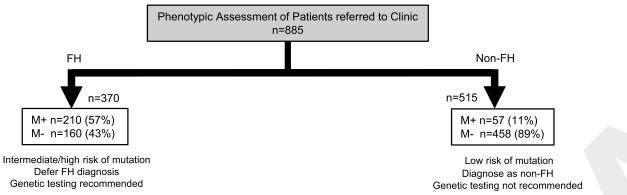
(A) Dutch Lipid Clinic Network Phenotype



(B) Simon Broome Phenotype



(C) Make Early Diagnosis to Prevent Early Deaths Phenotype



(D) American Heart Association Phenotype