J Lipid Atheroscler. 2024 Jan;13(1):29-40
https://doi.org/10.12997/jla.2024.13.1.29
pISSN 2287-2892·eISSN 2288-2561

JLA Journal of Lipid and Atherosclerosis

Original Article

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Association of Clinical Characteristics With Familial Hypercholesterolaemia Variants in a Lipid Clinic Setting: A Case-Control Study

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OPEN ACCESS

Received: Jul 2, 2023
Revised: Sep 5, 2023
Accepted: Sep 22, 2023
Published online: Oct 26, 2023

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Funding
None.

Conflict of Interest
The authors have no conflicts of interest to declare.

Data Availability Statement
The participants of this study did not give written consent for their data to be shared publicly, so due to the sensitive nature of the research supporting data is not available.

ABSTRACT

Objective: Familial hypercholesterolaemia (FH) variant positive subjects have over double the cardiovascular risk of low-density-lipoprotein-cholesterol (LDL-C) matched controls. It is desirable to optimise FH variant detection.
Methods: We identified 213 subjects with FH gene panel reports (*LDLR*, *APOB*, *PCSK9*, and *APOE*) based on total cholesterol >310 mg/dL; excluding triglycerides >400 mg/dL, cascade screening, and patients without pre-treatment LDL-C recorded. Demographic, clinical and lipid parameters were recorded.
Results: A 31/213 (14.6%) patients had pathogenic or likely pathogenic FH variants. 10/213 (4.7%) had variants of uncertain significance. Compared with patients without FH variants, patients with FH variants were younger (median age, 39 years vs. 48 years), had more tendon xanthomata (25.0% vs. 11.4%), greater proportion of first degree relatives with total cholesterol >95th percentile (40.6% vs. 16.5%), higher LDL-C (median, 271 mg/dL vs. 236 mg/dL), and lower triglycerides (median, 115 mg/dL vs. 159 mg/dL). The Besseling et al. model (c-statistic 0.798) improved FH variant discrimination over Friedewald LDL-C (c-statistic 0.724), however, Dutch Lipid Clinic Network Score (DLCNS) did not (c-statistic 0.665). Sampson LDL-C (c-statistic 0.734) had similar discrimination to Friedewald.
Conclusion: Although tendon xanthomata and first degree relatives with high total cholesterol >95th percentile were associated with FH variants, DLCNS or Simon Broome criteria did not improve FH detection over LDL-C. Sampson LDL-C did not significantly improve discrimination over Friedewald. Although lower triglycerides and younger age of presentation are positively associated with presence of FH variants, this information is not commonly used in FH detection algorithms apart from Besseling et al.

Keywords: Hyperlipoproteinemia type II; LDLR protein, human; PCSK9 protein, human; APOB protein, human; Dutch Lipid Clinic Network Score; Besseling

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Author Contributions

Conceptualization: Li B; Data curation: Li B, Laurie A, Reid N, Leath M, Chan H, Florkowski C; Formal analysis: Li B; Investigation: Chan H; Methodology: Li B, King R; Supervision: Florkowski C; Writing - original draft: Li B; Writing - review & editing: Li B, Laurie A, Reid N, Leath M, King R, Chan H, Florkowski C.

INTRODUCTION

Familial hypercholesterolaemia (FH) is characterised by marked elevation of low-density-lipoprotein-cholesterol (LDL-C). FH is usually inherited in an autosomal dominant manner, with variants in *LDLR*, *PCSK9* or *APOB*, or variants in *APOE*, which may be either autosomal dominant or recessive.[1]

There is considerable overlap of LDL-C between patients with FH and polygenic hypercholesterolaemia.[2,3] However, FH variant positive subjects have over double the cardiovascular risk of LDL-C matched controls.[2] The identification of FH variants warrants more intensive lipid lowering including higher doses of statins, especially with the availability of newer agents such as ezetimibe and proprotein convertase subtilisin/kexin type 9 inhibitors if required to reach LDL-C treatment targets. For best targeting of resources, it is desirable to assign an optimal strategy for variant detection in probands, which in turn will enable better cascade screening.

Different clinical criteria exist for diagnosis of FH, including the Dutch Lipid Clinic Network Score (DLCNS) or Simon Broome Criteria.[4-6] Recently, there have been several studies evaluating the discrimination of different clinical criteria, and proposed new models to improve the yield of genetic testing for FH.[1,7,8] A 2013 consensus statement for FH by the European Atherosclerosis Society recommends identifying probands by plasma total cholesterol ≥310 mg/dL (8 mmol/L),[9] which was the criteria used for most FH gene panels ordered in our institution during this study.

This retrospective case-control study was performed to determine associations between clinical criteria and FH gene variant positivity based on exon sequencing of *LDLR*, *PCSK9*, *APOB*, and *APOE*.

MATERIALS AND METHODS

Patients with FH gene panel reports (*LDLR*, *APOB*, *PCSK9*, and *APOE*) in our database requested from the Lipid Clinic of Christchurch Hospital, New Zealand, between 12 February 2019 to 6 July 2021 were evaluated.

Out of 261 patients seen in Lipid Clinic, patients were excluded if they were duplicates (n=3), had clinical notes indicating hypertriglyceridaemia or suspicion of familial combined hyperlipidaemia (n=15), triglycerides >400 mg/dL (4.5 mmol/L) prohibiting use of Friedewald LDL-C calculation (n=2), cascade screening based on known familial variants (n=14), cascade screening based on clinical diagnosis of FH in first degree relatives (n=6) and patients without pre-treatment cholesterol or estimate of such in clinical notes or test result database (n=3). Out of the remaining 218 patients, the pre-treatment total cholesterol but no other parameters were noted in five patients, which were excluded in any analyses requiring high-density-lipoprotein-cholesterol (HDL-C), LDL-C, non-HDL-C or triglycerides. Patients who already had a known variant in *LDLR* based on prior testing and who had FH panel performed to look for a second variant were not excluded, as the prior knowledge of the variant was not expected to significantly alter most variables considered in the analysis. Children were not excluded but were a small portion of the overall cohort (4/218 with age <16 years; 3/218 aged 16–<18 years).

https://doi.org/10.12997/jla.2024.13.1.29

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Demographic, clinical and lipid parameters corresponding to pre-treatment lipid profiles with the highest LDL-C by Friedewald formula before or up to the date of the FH panel were recorded for all patients retrospectively in the database. To evaluate whether the Sampson equation would substantially improve diagnostic yield,[10] the LDL-C by Sampson equation was calculated. Peak lipoprotein (a) levels measured by Abbott ARCHITECT Ci2000 (Abbott Diagnostics) before or up to the date of the FH panel were also recorded. Clinical parameters for calculation of DLCNS and Simon Broome criteria scores before the incorporation of gene panel data were based on the new patient nurse assessment and initial physician assessments if present.

FH gene panels were performed using an Ampliseq-for-Illumina next generation sequencing custom panel on an Illumina MiSeq instrument, targeting *LDLR*, *APOB* (exon 26), *PCSK9*, *APOE*, and *LDLRAP1*. The *LDLR* gene was also analysed by Multiplex ligation-dependent probe amplification to detect exon size copy number variants. Variants in *LDLR*, *APOB*, *PCSK9*, and *APOE* considered likely pathogenic and pathogenic were considered positive. Variants of uncertain significance (VUS) were considered separately and tabulated in baseline statistics, but excluded from calculations of discrimination. Variants were curated according to the ClinGen Familial Hypercholesterolemia Expert Panel Specifications to the American College of Medical Genetics and Genomics and the Association of Molecular Pathology Variant Interpretation Guidelines Version 1.2.

Ethnicity data was initially combined into broad categories of European, Asian, Maori, and Other. Since initial analyses of the database indicated higher FH variant detection in Asians, contrary to previously published data, Asian ethnicities were later divided into Filipino, Indian, and Other Asian, while European ethnicities were divided into South African European, Dutch, and Other European, to elucidate specific ethnicities driving this effect. Patients with multiple ethnicities recorded including European and other ethnicities were classified as European, with the exception of Maori ethnicity, most of whom were a combination of Maori and European, and classified as Maori.

Two separate definitions were tested for premature cardiovascular disease (CVD). The New Zealand guidelines for Cardiovascular Disease Risk Assessment and Management for Primary Care in 2018 defined family history of premature CVD as having a first-degree relative hospitalised or having died due to a heart attack or stroke before age 50 years.[11] However, based on European Society of Cardiology (ESC) guidelines, the DLCNS criteria and Besseling et al.[1] consider premature CVD as occurring in patients before age 55 years for men, and 60 years for women.[4] Patients having a personal or family history satisfying the New Zealand criteria were considered to have “premature” personal or family history of CVD, while those satisfying ESC criteria but not the more stringent New Zealand criteria were considered to be “between definitions”, and those with personal or family history of CVD at older ages were considered to have “non-premature” CVD.

Since the total cholesterol of first-degree relatives was often recorded in clinical notes if known but not LDL-C, for the DLCNS criteria of first degree relative with LDL-C above the 95th percentile, a total cholesterol above the 95th percentile based on age and sex was used as a surrogate. 95th percentiles for total cholesterol were based on a local study from Christchurch blood donors in 1981, thought to more accurately reflect untreated cholesterol levels in the local population, generally higher than other 95th percentiles published contemporarily from other jurisdictions (**Supplementary Table 1**).[12]

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As per New Zealand guidelines, diabetes was defined as either glycated haemoglobin (HbA1c) ≥50 mmol/mol (6.7%), fasting plasma glucose ≥126 mg/dL (7.0 mmol/L) or 2-hour plasma glucose on glucose tolerance test ≥200 mg/dL (11.1 mmol/L) on at least two tests if asymptomatic, or on one test if symptomatic. Pre-diabetes was defined as HbA1c between 41–49 mmol/mol (5.9%–6.6%), fasting plasma glucose from 110–125 mg/dL (6.1–6.9 mmol/L), or 2-hour plasma glucose on glucose tolerance test 140–199 mg/dL (7.8–11.0 mmol/L) on at least one test.

Information about statin use and diabetes were input as at the visit just prior to the performing of the FH gene panel. DLCNS and Simon Broome criteria scores were calculated using information available without the mutation result. For the Besseling et al.[1] criteria, since the lipid profiles corresponding to peak untreated LDL-C were available for most patients, these were used instead of adjusting for the effects of lipid lowering agents, which was done in the original model. Information regarding smoking status, hypertension, arcus cornealis at any age or before 45 years and tendon xanthomata was also collated. For any missing data for categorical variables, the mode of such variable was used in the analysis. Differences between FH variant positive, negative and VUS patients in continuous variables were tested using a Kruskal-Wallis test. Differences in categorical variables were tested using a Fisher's exact test.

Receiver operating characteristic (ROC) curves were generated to evaluate discrimination of candidate predictors for FH variants, with in-sample sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) calculated. Particular attention was paid to cut-offs chosen with sensitivity of 1.00 while maximising specificity, aiming to rationalise testing as much as possible without compromising detection of FH variants. Statistical analyses were performed using R version 4.1.2. This study was performed in accordance with the Declaration of Helsinki. Signed informed consent was received from all patients for this study. The institutional ethics committee waived the requirement for full ethics approval as all testing was performed as part of routine clinical care, and data were presented in a de-identified manner.

RESULTS

Of 218 patients who met the inclusion criteria, 32 had a pathogenic or likely pathogenic FH variant (14.7%) while 10 had a VUS (4.6%). A 98/218 patients (45.0%) were male, with median age of 47 years old (range, 5–68 years) at the time of lipid profile with peak LDL-C (**Table 1**). When solely considering the 213 patients with pre-treatment lipid profile recorded, 31 of these patients had a FH variant.

Compared with patients without FH variants, patients with FH variants were younger (median age, 39 years vs. 48 years), more had tendon xanthomata (25.0% vs. 11.4%), greater proportion of first degree relatives with total cholesterol >95th percentile (40.6% vs. 16.5%), and had higher LDL-C (median, 271 mg/dL [7.0 mmol/L] vs. 236 mg/dL [6.1 mmol/L]). There was no significant difference between Friedewald and Sampson equations for estimating LDL-C in either group or in discrimination for FH variants.

Those with FH variant had lower triglycerides (median, 115 mg/dL [1.3 mmol/L] vs. 159 mg/dL [1.8 mmol/L]) and there was a trend to lower HDL-C which did not meet statistical

https://doi.org/10.12997/jla.2024.13.1.29

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Table 1. Baseline demographics for total patient cohort

Variable	Median (IQR) or proportion (%)
Male sex	98/218 (45.0)
Age (yr)	47 (37–56)
Ethnicity	Other European 171/218 (78.4) Maori 11/218 (5.0) South African European 10/218 (4.6) Filipino 8/218 (3.7) Other Asian 7/218 (3.2) Indian 5/218 (2.3) Dutch 3/218 (1.4) Other 3/218 (1.4)
Cardiovascular disease	No 191/218 (87.6) Premature 15/218 (6.9) Between* 7/218 (3.2) Non-premature 5/218 (2.3)
Family history premature CVD	No 120/218 (55.0) Between* 31/218 (14.2) Yes 67/218 (30.7)
Smoking	No 148/218 (67.9) Ex-smoker 50/218 (22.9) Ex-smoker <12 mon 3/218 (1.4) Current smoker 17/218 (7.8)
Hypertension	49/218 (22.5)
Alcohol	176/218 (80.7)
Statin use	132/218 (60.6)
Diabetes	No 197/218 (90.4) Pre-diabetes 16/218 (7.3) Diabetes 5/218 (2.3)
Arcus cornealis	No 159/218 (72.9) At age <45 yr 14/218 (6.4) At older or unknown onset 45/218 (20.6)
Tendon xanthomata	32/218 (14.7)
First degree relative with total cholesterol >95th percentile	47/218 (21.6)
Total cholesterol (mg/dL [mmol/L])	333 (306–367), (8.6 [7.9–9.5])
Triglycerides (mg/dL [mmol/L])	151 (106–195), (1.7 [1.2–2.2])
HDL-C (mg/dL [mmol/L])	55 (47–67), (1.41 [1.21–1.74])
LDL-C (Friedewald), (mg/dL [mmol/L])	244 (209–271), (6.3 [5.4–7.0])
Lipoprotein (a) (mg/dL)	23.4 (8.4–83.3)
DLCNS score pretest	5 (3–8)
DLCNS category pretest	No 18/213 (8.5) Possible 109/213 (51.2) Probable 39/213 (18.3) Definite 47/213 (22.1)
Simon Broome classification pretest	No 85/218 (39.0) Probable 101/218 (46.3) Definite 32/218 (14.7)
FH probability (Besseling et al.[1])	0.83 (0.67–0.92)
Any FH variant	Pathogenic or likely pathogenic 32/218 (14.7) VUS 10/218 (4.6)
LDLR variant	Pathogenic or likely pathogenic 26/218 (11.9) VUS 5/218 (2.3)
PCSK9 variant	Pathogenic or likely pathogenic 3/218 (1.4) VUS 2/218 (0.9)
APOB variant	Pathogenic or likely pathogenic 1/218 (0.5) VUS 2/218 (0.9)
APOE variant	Pathogenic or likely pathogenic 2/218 (0.9) VUS 1/218 (0.5)

IQR, interquartile range; CVD, cardiovascular disease; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; DLCNS, Dutch Lipid Clinic Network Score; FH, familial hypercholesterolaemia.
*Between definitions of premature CVD between different guidelines, onset 50–54 years old in males or 50–59 years old in females.

https://doi.org/10.12997/jla.2024.13.1.29

JLA Journal of Lipid and Atherosclerosis

significance (median, 53 mg/dL [1.36 mmol/L] vs. 55 mg/dL [1.43 mmol/L]). There appeared to be significant differences by ethnicity and smoking status, with fewer patients with FH variants being current smokers. DLCNS and Simon Broome classifications before incorporating variant information more likely indicated FH in patients eventually found to have variants, and the probability of FH by the model of Besseling et al.[1] indicated higher likelihood of FH in patients found to have a variant. However, there did not appear to be a significant association of FH variants with either personal or family history of CVD, arcus cornealis, or lipoprotein (a) (**Table 2**). The proportions of patients without FH variants, VUS and likely pathogenic or pathogenic FH variants based on selected variables are also presented graphically (**Fig. 1** in mg/dL, **Supplementary Fig. 1** in mmol/L).

Of the standard lipid test parameters, LDL-C estimation by Friedewald, with ROC area under the curve (AUC) 0.724, or Sampson equation (AUC, 0.734) had the best discrimination for identifying FH variants, while total cholesterol (AUC, 0.623) and non-HDL-C (AUC, 0.659) had poorer discrimination. DLCNS before incorporating variant data (AUC, 0.665) did not have better discrimination than LDL-C alone, while the model of Besseling et al.[1] (AUC, 0.798) had modest performance improvement over LDL-C alone in identifying FH variants (**Fig. 2**).

The within-sample sensitivity, specificity, PPV and NPV of selected parameters shown to have statistically significant odds ratios for FH variants at different cut offs were calculated. LDL-C >194 mg/dL (5.0 mmol/L) had a sensitivity of 1.00 for detecting FH variants and specificity 0.18, yielding PPV 0.18 and NPV 1.00. Tendon xanthomata and first-degree relative with total cholesterol >95th percentile had modest sensitivity (0.25 and 0.41) and PPV (0.29 and 0.31) despite the high prevalence of FH variants in the study population. Statistics were also displayed for triglycerides <151 mg/dL (1.7 mmol/L) and HDL-C <77 mg/dL (2.00 mmol/L), though the specificity and PPV for these may be an overestimate due to the nature of the cohort.

Though Besseling et al.[1] initially advised that patients should have FH genotyping performed with FH probabilities at least 0.30 by their model, only 2/213 patients with untreated lipid panel in our cohort had a FH probability less than 0.30 by the Besseling et al.[1] model as applied in the dataset. However, a Besseling et al.[1] FH probability ≥0.450 in the dataset still maintained sensitivity 1.00, with specificity 0.10, PPV 0.17 and NPV 1.00 (**Table 3**).

DISCUSSION

Due to the substantial overlap in LDL-C between patients with FH variants and patients with polygenic hypercholesterolaemia, the accurate diagnosis of FH without genetic testing is difficult. In this study, the use of DLCNS or Simon Broome criteria did not result in better discrimination for FH variants than LDL-C alone. In addition, the use of Sampson equation for LDL-C did not result in significant improvement in identifying FH variants. However, using the Besseling et al.[1] model may modestly improve discrimination for FH variants, if an appropriate cut-off is selected.

In patients with suspected FH variants, the PPV of Definite FH by DLCNS ranges from 0.35 in a study from Korea to 0.82 in the United Kingdom (UK), and Definite FH by Simon Broome criteria varies from 0.37 in Korea to 0.73 in the UK.[7,8] Our PPV of 0.29 to 0.31 for Definite FH by either criteria falls towards the lower end of these observations.

https://doi.org/10.12997/jla.2024.13.1.29

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Table 2. Baseline statistics by variant status median (IQR) or proportion (%)

Variable	No FH variant	VUS	FH variant +	*p*-value
Male sex	78/176 (44.3)	4/10 (40)	16/32 (50.0)	0.791
Age (yr)	48 (39–56)	48 (40–53)	39 (24–49)	0.022*
Ethnicity	Other European 145/176 (82.4)	Other European 9/10 (90)	Other European 17/32 (53.1)	0.010*
	Maori 8/176 (4.5)	Maori 0/10 (0)	Maori 3/32 (9.4)	
	South African European 7/176 (4.0)	South African European 0/10 (0)	South African European 3/32 (9.4)	
	Filipino 3/176 (1.7)	Filipino 0/10 (0)	Filipino 5/32 (15.6)	
	Other Asian 5/176 (2.8)	Other Asian 0/10 (0)	Other Asian 2/32 (4.7)	
	Indian 2/176 (1.1)	Indian 1/10 (10)	Indian 2/32 (6.3)	
	Dutch 3/176 (1.7)	Dutch 0/10 (0)	Dutch 0/32 (0.0)	
	Other 3/176 (1.7)	Other 0/10 (0)	Other 0/32 (0.0)	
Cardiovascular disease	No 154/176 (87.5)	No 8/10 (80)	No 29/32 (90.6)	0.456
	Premature 13/176 (7.4)	Premature 1/10 (10)	Premature 1/32 (3.1)	
	Between 6/176 (3.4)	Between 0/10 (0)	Between 1/32 (3.1)	
	Non-premature 3/176 (1.7)	Non-premature 1/10 (10)	Non-premature 1/32 (3.1)	
Family history premature CVD	No 97/176 (55.1)	No 6/10 (60)	No 17/32 (53.1)	0.776
	Between definitions 27/176 (15.3)	Between definitions 0/10 (0)	Between definitions 4/32 (12.5)	
	Yes 52/176 (29.5)	Yes 4/10 (40)	Yes 11/32 (34.4)	
Smoking	No 113/176 (64.2)	No 6/10 (60)	No 29/32 (90.6)	0.018*
	Ex-smoker 44/176 (25.0)	Ex-smoker 4/10 (40)	Ex-smoker 2/32 (6.3)	
	Ex-smoker <12 mon 2/176 (1.1)	Ex-smoker <12 mon 0/10 (0)	Ex-smoker <12 mon 1/32 (3.1)	
	Current smoker 17/176 (9.7)	Current smoker 0/10 (0)	Current smoker 0/32 (0.0)	
Alcohol use	147/176 (83.5)	8/10 (80)	21/32 (65.6)	0.054
Statin use	107/176 (60.8)	6/10 (60)	19/32 (59.4)	1
Diabetes	No 162/176 (92.0)	No 8/10 (80)	No 27/32 (84.4)	0.023*
	Pre-diabetes 12/176 (6.8)	Pre-diabetes 0/10 (0)	Pre-diabetes 4/32 (12.5)	
	Diabetes 2/176 (1.1)	Diabetes 2/10 (20)	Diabetes 1/32 (3.1)	
Hypertension	43/176 (24.4)	3/10 (30)	3/32 (9.4)	0.121
Arcus cornealis	No 129/176 (73.3)	No 7/10 (70)	No 23/32 (71.9)	0.763
	<45 yr 10/176 (5.7)	<45 yr 1/10 (10)	<45 yr 3/32 (9.4)	
	Older or onset unknown 37/176 (21.0)	Older or onset unknown 2/10 (20)	Older or onset unknown 6/32 (18.8)	
Tendon xanthomata	20/176 (11.4)	4/10 (40)	8/32 (25.0)	0.009†
First degree relative with total cholesterol >95th percentile	29/176 (16.5)	5/10 (50)	13/32 (40.6)	<0.001‡
Fasting status	Fasting 119/176 (67.6)	Fasting 7/10 (70)	Fasting 21/32 (65.6)	0.809
	Non-fasting 26/176 (14.7)	Non-fasting 2/10 (20)	Non-fasting 7/32 (21.9)	
	Not stated 31/176 (17.6)	Not stated 1/10 (10)	Not stated 4/32 (12.5)	
Total cholesterol (mg/dL [mmol/L])	333 (306–360), (8.6 [7.9–9.3])	336 (321–379), (8.7 [8.3–9.8])	348 (317–394), (9.0 [8.2–10.2])	0.068
Triglycerides (mg/dL [mmol/L])	159 (115–204), (1.8 [1.3–2.3])	159 (142–204), (1.8 [1.6–2.3])	115 (89–159), (1.3 [1.0–1.8])	0.002†
HDL-C (mg/dL [mmol/L])	55 (48–68), (1.43 [1.24–1.76])	50 (41–63), (1.29 [1.05–1.64])	53 (42–67), (1.36 [1.09–1.73])	0.260
LDL-C (Friedewald), (mg/dL [mmol/L])	236 (205–263), (6.1 [5.3–6.8])	248 (232–278), (6.4 [6.0–7.2])	271 (244–309), (7.0 [6.3–8.0])	<0.001‡
LDL-C (Sampson) (mg/dL [mmol/L])	236 (209–263), (6.1 [5.4–6.8])	251 (232–271), (6.5 [6.0–7.0])	275 (248–313), (7.1 [6.4–8.1])	<0.001‡
Cholesterol:HDL-C ratio	5.9 (4.7–6.9)	6.8 (5.2–9.5)	6.9 (5.4–7.9)	0.018*
Non-HDL-C (mg/dL [mmol/L])	271 (240–298), (7.0 [6.2–7.7])	275 (259–333), (7.1 [6.7–8.6])	286 (267–336), (7.4 [6.9–8.7])	0.015*
Lipoprotein (a) (mg/dL)	22.6 (8.5–84.5)	16.8 (4.0–72.7)	24.4 (9.7–45.1)	0.585
DLCNS score pretest	4 (3–6)	9 (5–11)	6 (4–11)	<0.001‡
DLCNS category pretest	No 18/172 (10.5)	No 0/10 (0)	No 0/31 (0.0)	0.009†
	Possible 93/172 (54.1)	Possible 3/10 (30)	Possible 13/31 (41.9)	
	Probable 32/172 (18.6)	Probable 2/10 (20)	Probable 5/31 (16.1)	
	Definite 29/172 (16.9)	Definite 5/10 (50)	Definite 13/31 (41.9)	
Family history cholesterol >290 mg/dL (7.5 mmol/L)	45/176 (25.6)	5/10 (50)	14/32 (43.8)	0.033*
Simon Broome classification pretest	No 77/176 (43.8)	No 2/10 (20)	No 6/32 (18.8)	0.006†
	Probable 79/176 (44.9)	Probable 4/10 (40)	Probable 18/32 (56.3)	
	Definite 20/176 (11.4)	Definite 4/10 (40)	Definite 8/32 (25.0)	
FH probability (Besseling et al.[1])	0.79 (0.64–0.90)	0.89 (0.75–0.93)	0.96 (0.88–0.98)	<0.001‡

IQR, interquartile range; FH, familial hypercholesterolaemia; VUS, variants of uncertain significance; CVD, cardiovascular disease; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; DLCNS, Dutch Lipid Clinic Network Score.
*$p < 0.05$, †$p < 0.01$, ‡$p < 0.001$.

https://doi.org/10.12997/jla.2024.13.1.29

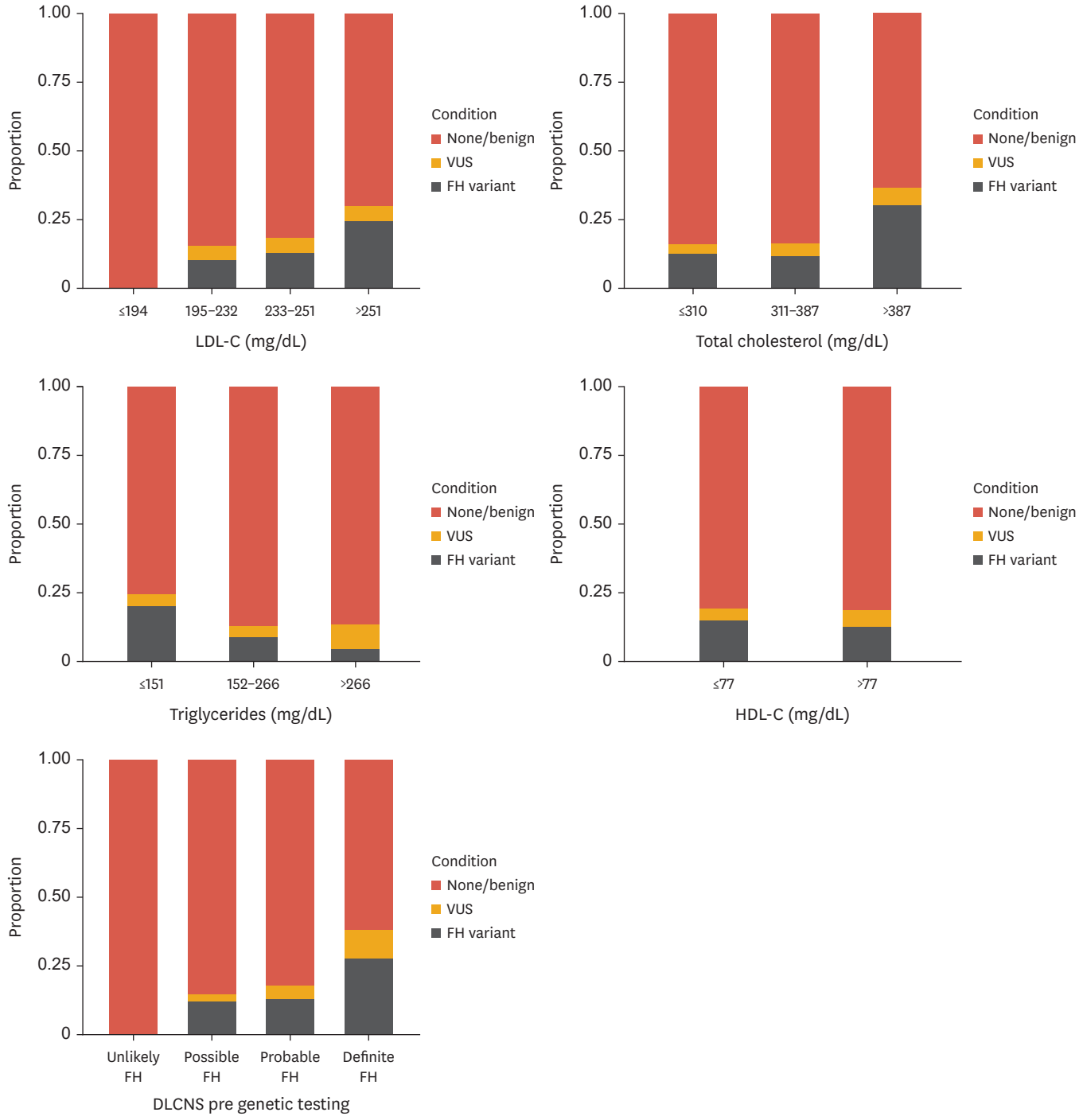

Fig. 1. Proportions of patients with no identified FH variant/benign, VUS, and likely pathogenic or pathogenic FH variant given selected characteristics (mg/dL units).
VUS, variants of uncertain significance; FH, familial hypercholesterolaemia; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; DLCNS, Dutch Lipid Clinic Network Score.

The variation in PPV for DLCNS or Simon Broome criteria between studies may be attributable to differences in FH variants around the world, leading to different pre-test probabilities for FH variants. A review of worldwide FH prevalence in the general population found the prevalence of FH to be between 1/1,000 and 1/500 in Korea, while the prevalence was between 1/332 and 1/250 in the UK.[13] However, directly obtained prevalence data for

https://doi.org/10.12997/jla.2024.13.1.29

JLA Journal of Lipid and Atherosclerosis

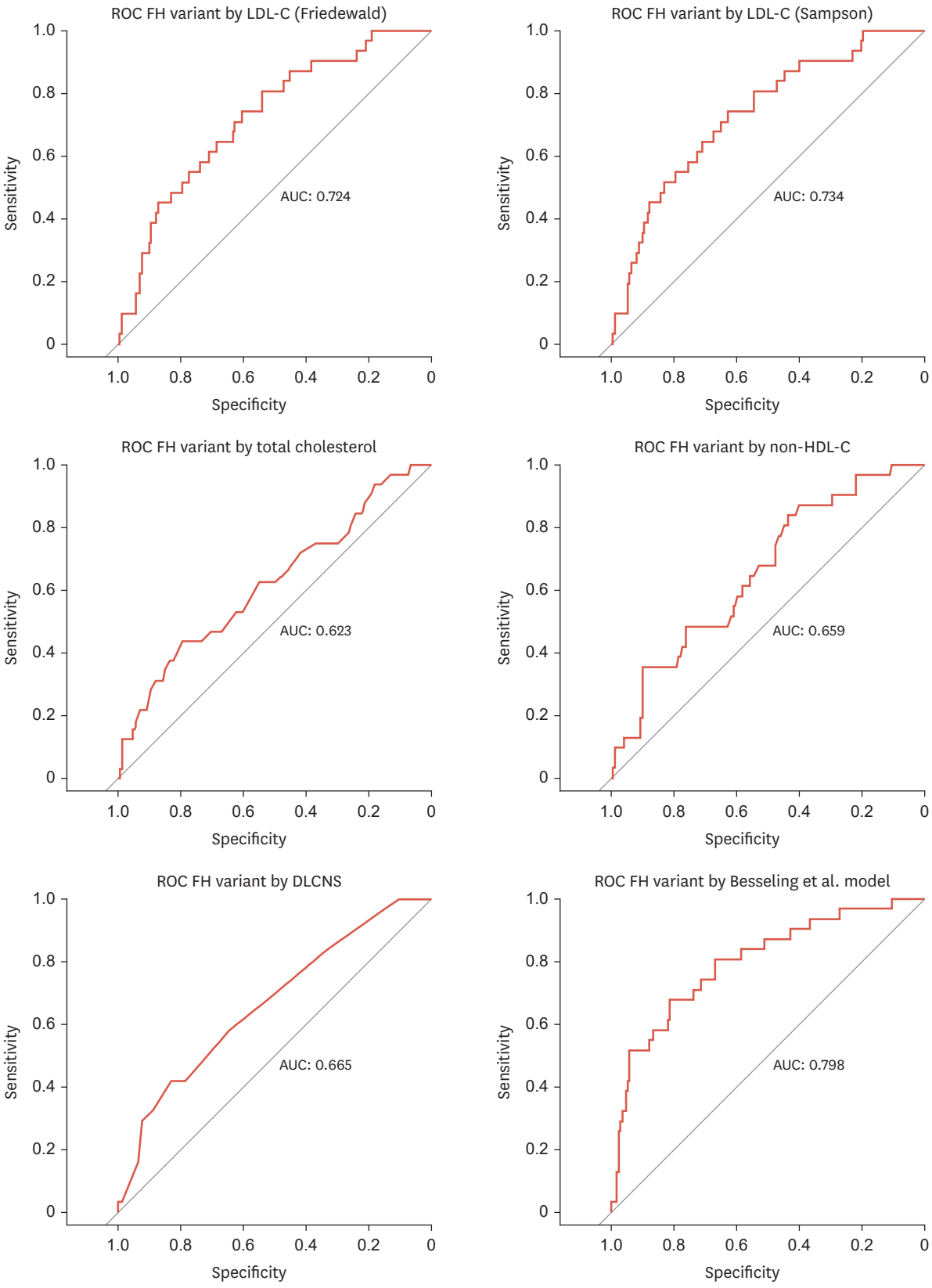

Fig. 2. ROC curves for LDL-C (Friedewald), LDL-C (Sampson), total cholesterol, non-HDL-C, DLCNS (pre-mutation) and Besseling et al.[1]
ROC, receiver operating characteristic; FH, familial hypercholesterolaemia; LDL-C, low density lipoprotein cholesterol; AUC, area under the curve; HDL-C, high density lipoprotein cholesterol; DLCNS, Dutch Lipid Clinic Network Score.

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Table 3. SN, SP, PPV, and NPV for selected baseline characteristics for FH variants

Variable	SN (95% CI)	SP (95% CI)	PPV (95% CI)	NPV (95% CI)
Tendon xanthomata	0.25 (0.11–0.43)	0.89 (0.83–0.93)	0.29 (0.13–0.49)	0.87 (0.81–0.91)
1st degree relative with TC >95th percentile	0.41 (0.24–0.59)	0.84 (0.77–0.89)	0.31 (0.18–0.47)	0.89 (0.83–0.93)
TC >310 mg/dL (8.0 mmol/L)	0.75 (0.57–0.89)	0.30 (0.23–0.37)	0.16 (0.11–0.23)	0.87 (0.76–0.94)
TC >387 mg/dL (10.0 mmol/L)	0.31 (0.16–0.50)	0.88 (0.82–0.92)	0.32 (0.17–0.51)	0.88 (0.82–0.92)
LDL-C >194 mg/dL (5.0 mmol/L)	1.00 (0.89–1.00)	0.18 (0.13–0.25)	0.18 (0.13–0.25)	1.00 (0.89–1.00)
LDL-C >232 mg/dL (6.0 mmol/L)	0.81 (0.63–0.93)	0.47 (0.39–0.55)	0.22 (0.14–0.30)	0.93 (0.86–0.97)
LDL-C >252 mg/dL (6.5 mmol/L)	0.65 (0.45–0.81)	0.66 (0.58–0.73)	0.25 (0.16–0.36)	0.91 (0.85–0.95)
Triglycerides <151 mg/dL (1.7 mmol/L)	0.74 (0.55–0.88)	0.58 (0.50–0.66)	0.24 (0.16–0.34)	0.93 (0.86–0.97)
HDL-C <77 mg/dL (2.00 mmol/L)	0.87 (0.70–0.96)	0.16 (0.11–0.22)	0.16 (0.11–0.22)	0.87 (0.70–0.96)
DLCNS “definite”	0.42 (0.25–0.61)	0.83 (0.77–0.88)	0.31 (0.18–0.47)	0.89 (0.83–0.93)
DLCNS “definite or probable”	0.58 (0.39–0.75)	0.65 (0.57–0.72)	0.23 (0.14–0.34)	0.90 (0.83–0.94)
DLCNS “definite, probable or possible”	1.00 (0.89–1.00)	0.10 (0.06–0.16)	0.17 (0.12–0.23)	1.00 (0.81–1.00)
Simon Broome “definite”	0.25 (0.11–0.43)	0.89 (0.83–0.93)	0.29 (0.13–0.49)	0.87 (0.81–0.91)
Simon Broome “definite or probable”	0.81 (0.64–0.93)	0.44 (0.36–0.51)	0.21 (0.14–0.29)	0.93 (0.85–0.97)
Besseling et al.[1] P(mutation) ≥0.450	1.00 (0.89–1.00)	0.10 (0.06–0.16)	0.17 (0.12–0.23)	1.00 (0.81–1.00)
Besseling et al.[1] P(mutation) ≥0.650	0.97 (0.83–1.00)	0.26 (0.20–0.33)	0.19 (0.13–0.26)	0.98 (0.88–1.00)

SN, sensitivity; SP, specificity; PPV, positive predictive value; NPV, negative predictive value; FH, familial hypercholesterolaemia; CI, confidence interval; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; DLCNS, Dutch Lipid Clinic Network Score.

FH in New Zealand is not available. The PPV may also depend on whether peak LDL-C or a random baseline LDL-C is used, where using peak untreated LDL-C instead of a random or trough untreated LDL-C is expected to result in higher scores for patients and lower PPV.

Although higher LDL-C is widely known to be associated with FH variants, this study demonstrates that patients with FH variants have lower triglycerides and younger age. This is consistent with other data from both lipid clinics or hospitals and in cascade screening populations,[1,7,8] though apart from the Besseling et al.[1] model, this information is not widely used to optimise the yield of FH variant testing. As such, at least some of the increased discrimination of the Besseling et al.[1] model compared to DLCNS or Simon Broome criteria is presumably due to considering the inverse associations between triglycerides and age with presence of FH variants. In addition, the use of a large dataset with both clinical parameters and genetic sequencing information in the cascade screening cohort underlying the Besseling et al.[1] model likely enabled better fine tuning of coefficients in the model, compared to the likely more empirically derived DLCNS or Simon Broome criteria. In order to prevent CVD, it is desirable to identify FH variants to better target lipid lowering therapy in younger patients before the development of atherosclerosis. However, younger patients typically have lower LDL-C,[12,14] and tendon xanthomata forming part of DLCNS or Simon Broome criteria require time to develop. As such, clinicians should have a lower threshold for FH variant testing in younger patients.

Although a cut-off of FH probability ≥0.30 by the Besseling et al.[1] model was suggested to trigger FH genotyping, only two patients in this study cohort did not fulfill this criteria. A sensitivity of 100% was still maintained by increasing the cut-off to 0.45. This may be due to the lipid profile corresponding to the peak untreated LDL-C being used in the analysis, rather than a random LDL-C baseline, increasing the calculated probability of FH by Besseling et al.[1] when not calibrated to the difference in inputs. Furthermore, Besseling et al.[1] corrected for expected differences in LDL-C if lipid lowering therapy was present on the lipid profile being used for analysis, in contrast to this study which used untreated lipid profiles corresponding to peak LDL-C. Using correction equations may be required for larger studies but does not consider differences in adherence, pharmacodynamics or pharmacokinetics between individuals, though may have affected the comparison in this study.

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Consistent with data from UK but not Korea, presence of tendon xanthomata was significantly associated with FH variants.[7,8] The 95th percentile of total cholesterol from the local population in 1981 used in this study, approximately 8.2 mmol/L if aged 50 years or older, is significantly higher than published in other sources.[12,14] Since LDL-C has a greater discrimination for FH variants than total cholesterol, a family history of LDL-C >95th percentile may be more discriminatory than a total cholesterol >95th percentile if this information is available, though this data was not readily available for this study.

The prevalence of FH is enriched in patients with both premature and non-premature coronary artery disease (CAD) compared to the general population, and noted on the development cohort of Besseling et al.[1] in a large cascade screening population.[13] However, consistent with other studies in hyperlipidaemic patients, this study did not find a statistically significant difference in either premature or non-premature CAD in either patients or first-degree relatives in patients with or without FH variants.[7,8] It is possible that most of the effect of information carried by premature CAD for FH variants is contained in the lipid profile itself. Arcus status was also not significantly associated with FH variant status, though this study was not powered to detect a difference for patients with arcus cornealis at age <45 years old.

Contrary to prior studies indicating a lower prevalence of FH in Asian patients,[13] this study indicated a greater odds ratio for FH variants in Asian patients compared to European patients. When different Asian ethnicities were separated, this was largely driven by a higher prevalence of FH in Filipino and Indian patients in the dataset. As there is no direct prevalence data for FH in the Philippines and India, it is unclear whether this reflects a truly increased prevalence of FH in Filipinos and Indians. However, this study was not powered to detect differences between ethnicities. Nevertheless, this study highlights the lack of prevalence data for FH in many countries around the world.

There are several limitations to this study. Though no patients with confirmed FH variant in our cohort had LDL-C ≤194 mg/dL (5.0 mmol/L), it is well recognised that a significant portion of patients with FH variants presents with LDL-C ≤194 mg/dL (5.0 mmol/L). As such, excluding patients for FH testing on this basis is not applicable for patients undergoing cascade screening with a known familial autosomal dominant FH variant, where the pre-test probability of FH is 50%. However, due to the low overall prevalence of FH in the community, the pre-test probability of a patient with LDL-C ≤194 mg/dL (5.0 mmol/L) having FH variants is low in the absence of cascade screening.

Although there was no minimum age requirement for this study, only a quarter of patients with FH variants were aged 30 years old or under, with an even smaller proportion of minors. Since LDL-C increases with age, lower cut-offs for LDL-C may be required in younger patients in practice.

In conclusion, the inverse association between increased age, triglycerides and trend to increased HDL-C with FH variant positivity is consistent with other literature but does not currently form part of FH diagnostic criteria. High LDL-C, tendon xanthomata, diabetes, first degree relative with cholesterol >95th percentile and certain ethnicities were also associated with FH variants in this study. Personal or family history of CVD and presence of corneal arcus did not appear to be strongly associated with presence of FH variants within this dataset, though this may be due to lack of statistical power. Their role in identifying patients for FH variant detection requires further study.

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SUPPLEMENTARY MATERIALS

Supplementary Table 1

Total cholesterol 95th percentiles (mg/dL [mmol/L]) in Christchurch blood donors (from Janus et al.[12] 1981)

Supplementary Fig. 1

Proportions of patients with no identified FH variant/benign, VUS, and likely pathogenic or pathogenic FH variant given selected characteristics (units in mmol/L).

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