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Mutations causative of familial hypercholesterolaemia: screening of 98 098 individuals from the Copenhagen General Population Study estimated a prevalence of 1 in 217

Marianne Benn[1,2,3]*, Gerald F. Watts[4], Anne Tybjærg-Hansen[2,3,5], and Børge G. Nordestgaard[2,3,6]

[1]Department of Clinical Biochemistry, Gentofte Hospital, Copenhagen University Hospital, Kildegårdsvej 28, DK-2900 Gentofte, Denmark; [2]The Copenhagen General Population Study, Herlev Hospital, Copenhagen University Hospital, Copenhagen, Denmark; [3]Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark; [4]School of Medicine and Pharmacology, Lipid Disorders Clinic, Cardiovascular Medicine, Royal Perth Hospital, University of Western Australia, Crawley, Australia; [5]Department of Clinical Biochemistry, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark; and [6]Department of Clinical Biochemistry, Herlev Hospital, Copenhagen University Hospital, Copenhagen, Denmark

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Aims Ideally, familial hypercholesterolaemia (FH) is diagnosed by testing for mutations that decrease the catabolism of low-density lipoprotein (LDL) cholesterol; however, genetic testing is not universally available. The aim of the present study was to assess the frequency and predictors of FH causing mutations in 98 098 participants from the general population, the Copenhagen General Population Study.

Methods and results We genotyped for *LDLR*[W23X;W66G;W556S] and *APOB*[R3500Q] accounting for 38.7% of pathogenic FH mutations in Copenhagen. Clinical FH assessment excluded mutation information. The prevalence of the four FH mutations was 0.18% (1:565), suggesting a total prevalence of FH mutations of 0.46% (1:217). Using the Dutch Lipid Clinic Network (DLCN) criteria, odds ratios for an FH mutation were 439 (95% CI: 170–1 138) for definite FH, 90 (53–152) for probable FH, and 18 (13–25) for possible FH vs. unlikely FH. Using the Simon Broome criteria, the odds ratio was 27 (20–36) for possible vs. unlikely FH, and using the Make Early Diagnosis to Prevent Early Death (MEDPED) criteria, 40 (28–58) for probable vs. unlikely FH. Odds ratios for an FH mutation were 17 (9–31) for LDL-cholesterol of 4–4.9 mmol/L, 69 (37–126) for LDL-cholesterol of 5–5.9 mmol/L, 132 (66–263) for LDL-cholesterol of 6–6.9 mmol/L, 264 (109–637) for LDL-cholesterol of 7–7.9 mmol/L, and 320 (129–798) for LDL-cholesterol above 7.9 mmol/L vs. LDL-cholesterol below 4 mmol/L. The most optimal threshold for LDL-cholesterol concentration to discriminate between mutation carriers and non-carriers was 4.4 mmol/L.

Conclusion Familial hypercholesterolaemia-causing mutations are estimated to occur in 1:217 in the general population and are best identified by a definite or probable phenotypic diagnosis of FH based on the DLCN criteria or an LDL-cholesterol above 4.4 mmol/L.

Keywords Familial hypercholesterolaemia • Coronary artery disease • Ischaemic heart disease • Myocardial infarction • Low-density lipoprotein • General population • *LDLR* mutation • *APOB* mutation

* Corresponding author. Tel: +45 3867 7494, Email: Marianne.Benn@regionh.dk

Introduction

Familial hypercholesterolemia (FH) is a common autosomal dominant genetic cause of premature coronary artery disease, due to lifelong elevated low-density lipoprotein (LDL) cholesterol concentrations.[1–3] Of FH causing mutations, >95% are in the LDL receptor, *LDLR*, 2–11% in apolipoprotein B, *APOB*, and <1% in proprotein convertase subtilisin/kexin type 9, *PCSK9*.[2–6] These mutations lead to decreased clearance of LDL-cholesterol from plasma and consequently increased total and LDL-cholesterol concentrations. If left untreated, heterozygotes for FH typically develop coronary artery disease before age 55, while homozygotes typically develop coronary artery disease very early in life and if untreated die before age 20.[2,5,7]

Familial hypercholesterolemia is underdiagnosed and undertreated in the general population,[3,8–11] and because coronary artery disease is the leading cause of death worldwide, many individuals with FH may be overlooked among the large number of individuals with coronary artery disease caused by more common risk factors, resulting in lost opportunities for preventing premature heart disease.[12]

Familial hypercholesterolemia is best diagnosed using clinical criteria in combination with genetic testing. However, genetic testing is not universally available. Nevertheless, for general practitioners, endocrinologists, and cardiologists, a pertinent question is how to identify patients with a high likelihood of carrying a mutation, to refer such patients to genetic testing or to improve the clinical diagnosis of FH. Certainly, knowledge of mutation carrier status or high likelihood thereof may help identify offspring at risk of coronary artery disease at an early age and thus enable better prevention.

The aim of the present study was (i) to determine the prevalence of the most common FH-causing mutations in an unselected community-based population and thereby estimate the total prevalence of FH mutations; (ii) to compare the ability of the three most commonly used diagnostic tools, the Dutch Lipid Clinic Network (DLCN) criteria, the Simon Broome criteria, and the Make Early Diagnosis to Prevent Early Death (MEDPED) criteria to predict FH mutation carrier status; (iii) to evaluate the ability of LDL-cholesterol concentrations to predict FH mutation carrier status; (iv) to examine the lipid phenotype of FH mutation carriers; and (v) to estimate the risk of coronary artery disease and myocardial infarction in FH mutation carriers compared with non-carriers.

Methods

The Copenhagen General Population Study

The Copenhagen General Population Study was initiated in 2003 with ongoing enrolment.[13] Individuals aged 20–100 were selected from the national Danish Civil Registration System to reflect the adult White Danish population of Danish descent. In total, 99 372 participants were included at time of analyses; however, as hypothyroidism may mimic FH with elevated LDL-cholesterol concentrations, 274 individuals were excluded due to hypothyroidism (thyroid-stimulating hormone >5 mIU/L and a total thyroxin <50 nmol/L and/or a total tri-iodothyronine <0.9 nmol/L), leaving 98 098 participants in the study. Data were obtained from a self-completed questionnaire that was reviewed together with an investigator on the day of attendance, from a brief physical examination, and from non-fasting venous blood samples. The study was approved by Copenhagen University Hospital and by a Danish ethical committee (H-KF-01-144/01), and was conducted according to the Declaration of Helsinki. Informed written consent was obtained from participants.

Diagnostic criteria for familial hypercholesterolaemia

The clinical diagnosis of FH was established using modified versions of the three commonly used clinical criteria (see Supplementary material online, *Table S1*). Modifications were used, because information on LDL-cholesterol in children and family and personal details of tendon xanthoma or corneal arcus were not recorded in our study.

Using the DLCN criteria,[14–16] a diagnosis of FH was considered definite if the total score was >8, probable if the score was 6–8, possible if the score was 3–5, and unlikely if the score was below 3 points (see Supplementary material online, *Table S1*). The score was calculated using points from the presence of family history of a first-degree relative with known premature (<55 years for men; <60 years for women) coronary artery disease or vascular disease, and/or a first-degree relative with known hypercholesterolaemia (1 point); the presence of personal history of premature coronary artery disease (ages as above, 2 points) or premature cerebral or peripheral vascular disease (ages as above, 1 point if not already 2 points for premature coronary artery disease); LDL-cholesterol >8.5 mmol/L (330 mg/dL, 8 points), 6.5–8.4 mmol/L (250–329 mg/dL, 5 points), 5.0–6.4 mmol/L (190–249 mg/dL, 3 points), and of 4.0–4.9 mmol/L (155–189 mg/dL, 1 point) (only highest LDL criteria generate points) (see Supplementary material online, *Table S1*); the presence of an *LDLR* W23X, W66G, or W556S or *APOB* R3500Q mutation (8 points). In the prediction of a mutation, information on mutation was excluded from the diagnosis.

Using the Simon Broome criteria,[17] a diagnosis of FH was considered definite if total cholesterol was >7.5 mmol/L (289 mg/dL) or LDL-cholesterol >4.9 mmol/L (189 mg/dL) in the presence of an *LDLR* W23X, W66G, or W556S, or *APOB* R3500Q mutation; and possible if total cholesterol was >7.5 mmol/L (289 mg/dL) or LDL-cholesterol >4.9 mmol/L (189 mg/dL), in the presence of a family history of a first-degree relative with premature myocardial infarction or with known hypercholesterolaemia (see Supplementary material online, *Table S1*). In the prediction of a mutation, information on mutation was excluded from the diagnosis, minimizing a definite diagnosis of FH as an outcome.

Using the MEDPED criteria,[18] a diagnosis of probable FH was considered for ages 20–29 years if total cholesterol was ≥7.5 mmol/L (290 mg/dL) or LDL-cholesterol ≥5.7 mmol/L (220 mg/dL); for ages 30–39 years, if total cholesterol was ≥8.8 mmol/L (340 mg/dL) or LDL-cholesterol ≥6.2 mmol/L (240 mg/dL); and for ages of 40 years and above, if total cholesterol was ≥9.3 mmol/L (360 mg/dL) or LDL-cholesterol ≥6.7 mmol/L (260 mg/dL) (see Supplementary material online, *Table S1*).

Coronary artery disease

A diagnosis of coronary artery disease, in the present study equivalent to ischaemic heart disease, comprised angina pectoris and myocardial infarction (WHO International Classification of Diseases, ICD8:410–414; ICD10:I20-I25), and information were collected from 1 January 1977 through 10 April 2013 by reviewing all hospital admissions and diagnoses entered in the national Danish Patient Registry and all causes of death entered in the national Danish Causes of Death Registry as described:[13,19] 8597 individuals had a diagnosis of coronary artery disease and of those 3227 had a diagnosis of myocardial infarction. No individuals were lost to follow-up.

Genotyping

LDLR W23X, W66G, and W556S and *APOB* R3500Q mutations were genotyped in all individuals by TaqMan assays (Applied Biosystems, Foster City, CA, USA). These four mutations account for 38.7% of FH mutations in the Copenhagen population (Anne Tybjærg-Hansen, from genetic testing of 142 index cases including full sequencing of *LDLR*, *PCSK9*, and the receptor-binding region of *APOB* and MLPA analysis of deletions/rearrangements in the *LDLR* gene at Copenhagen University Hospital) and all other previously reported mutations have very low prevalence, or are private mutations, in Denmark[20,21] (see Supplementary material online, *Table S2*). Genotypes were verified by sequencing of randomly selected individuals with each variant. There was 100% agreement between TaqMan and sequencing results.

Biochemical analyses

Non-fasting plasma concentrations of total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, and glucose were measured by standard enzymatic assays, while apolipoprotein B and apolipoprotein A-I were measured immunochemically (Thermo Fisher Scientific/Konelab). LDL-cholesterol was calculated using the Friedewald equation when plasma triglycerides were $\leq$4.0 mmol/L ($\leq$352 mg/dL) and measured by a direct enzymatic method at higher triglyceride concentrations (Thermo Fisher Scientific/Konelab). For the diagnostic classification, plasma LDL-cholesterol concentrations were multiplied by 1.43 in individuals receiving cholesterol-lowering medication, corresponding to an estimated 30% reduction in LDL-cholesterol.[22]

Other covariates

Body mass index was measured weight (kg) divided by measured height squared (m^2). Hypertension was systolic blood pressure $\geq$140 mmHg ($\geq$135 mmHg for diabetics), diastolic blood pressure $\geq$90 mmHg ($\geq$85 mmHg for diabetics), and/or use of antihypertensive medication. Metabolic syndrome was defined according to internationally agreed criteria.[23] Diabetes mellitus was self-reported diabetes, use of anti-diabetic medication, a non-fasting plasma glucose >11.0 mmol/L (>198 mg/dL), and/or hospitalized with diabetes (ICD8: 249–250; ICD10: E10-E11, E13-E14). Smokers were current smokers. Cholesterol-lowering medication was self-reported with >97% being statins; information on statin types or doses were not available.

Statistical analyses

Data were analysed using Stata/S.E.12.1. For genotypes, a deviation from the Hardy–Weinberg equilibrium was tested using a Pearson χ^2 test. Frequencies of non-lipid risk factors between mutation carriers and non-carriers, and frequencies of mutation carriers and non-carriers among the definitions of FH were compared by χ^2 tests.

The odds ratio in predicting the presence of an *LDLR* or *APOB* mutation compared with non-carriers was estimated for each FH diagnostic group using the DLCN, Simon Broome, and MEDPED criteria; for LDL-cholesterol levels by comparing individuals with a level above and below each of the criterion thresholds; and for LDL-cholesterol in categories of concentrations by logistic regression adjusted for age and gender.

With the use of receiver operating characteristic curve analysis, area under the curve was estimated as a measure of the ability of LDL-cholesterol concentration to discriminate between mutation carriers and non-carriers. By definition, random classification of carriers and non-carriers provides an area under the curve of 0.5, while perfect classification provides an area under the curve of 1.

Student's *t*-test examined differences in lipid, lipoprotein, and apolipoprotein concentrations between mutation carriers and non-carriers, off and on cholesterol-lowering medication. Risk of coronary artery disease and myocardial infarction for individuals with a mutation off and on cholesterol-lowering medication relative to non-carriers off cholesterol-lowering medication was estimated by logistic regression adjusted for age, gender, body mass index, metabolic syndrome, diabetes mellitus, hypertension, and smoking.

Results

Prevalence of familial hypercholesterolaemia mutation carriers

The prevalence of *LDLR* [W23X, W66G, W556S] or *APOB* [R3500Q] mutations combined was 0.18% (1:564), and 0.06% (1:1557) for *LDLR* and 0.11% (1:884) for *APOB* mutations separately. As these mutations account for 38.7% of all FH-causing mutations in genetic screening services in Copenhagen (see Supplementary material online, *Table S2*), this prevalence suggests a total prevalence of known FH-causing mutations of 0.46% (1:217); considered separately by gene, the corresponding numbers were 0.25% (1:395) for *LDLR* mutations and 0.85% (1:118) for *APOB* mutations. Genotypes did not deviate from the Hardy–Weinberg expectations (all $P >$ 0.86). The prevalence of coronary artery disease was 11% in mutation carriers compared with 9% in non-carriers. Frequency of non-lipid risk factors in mutation carriers and non-carriers is shown in Supplementary material online, *Table S3*.

Prediction of familial hypercholesterolaemia mutation carriers

Using the DLCN criteria, odds ratios for an FH mutation were 439 (95% CI: 170–1138) for definite FH, 90 (53–152) for probable FH, and 18 (13–25) for possible FH vs. unlikely FH (*Table 1*). Using the Simon Broome criteria, the odds ratio was 27 (20–36) for possible vs. unlikely FH. Finally, using the MEDPED criteria, the odds ratio was 40 (28–58) for probable vs. unlikely FH. Corresponding odds ratios for carrying an *LDLR* or *APOB* mutation are also shown separately in *Table 1*. The relative risk of carrying an FH mutation estimated for each of the criteria included in the DLCN, the Simon Broome, and the MEDPED criteria is shown in Supplementary material online, *Table S4*.

According to the DLCN criteria, 3.5% of carriers of one of the four FH mutations screened for were found among individuals classified as having definite FH, 11% among probable FH, 48% among possible FH, and 38% among individuals classified as unlikely FH (see Supplementary material online, *Table S5*). According to the Simon Broome criteria, 0% of mutation carries was found among individuals classified as definite FH, 52% among possible FH, and 48% among individuals classified as unlikely FH. According to the MEDPED criteria, 23% of mutation carriers were found among individuals classified as probable FH, and 77% among unlikely FH. In both the DLCN and Simon Broome criteria, the presence of a mutation alone is diagnostic for definite/probable FH; however, information on mutation carrier status was ignored in the above

Table 1 **Risk of having a low-density lipoprotein receptor or an apolipoprotein B gene mutation by the Dutch Lipid Clinic Network criteria, the Simon Broome criteria, and the Make Early Diagnosis to Prevent Early Death criteria in the Copenhagen General Population Study ignoring information on mutation carrier status during categorization**

		LDLR or *APOB* mutation			*LDLR* mutation			*APOB* mutation		
No. of participants		**No. of carriers**	**OR (95% CI)**	***P*-value**	**No. of carriers**	**OR (95% CI)**	***P*-value**	**No. of carriers**	**OR (95% CI)**	***P*-value**
Dutch Lipid Clinic Network criteria										
Unlikely FH	90 956	66	1 (Reference)		21	1 (Reference)		45	1 (Reference)	
Possible FH	6703	83	18 (13–25)	<0.001	23	15 (8–27)	<0.001	60	19 (13–28)	<0.001
Probable FH	316	19	90 (53–152)	<0.001	13	189 (94–382)	<0.001	6	40 (17–95)	<0.001
Definite FH	25	6	439 (170–1138)	<0.001	6	1327 (481–3664)	<0.001	0	—	<0.001
Simon Broome criteria										
Unlikely FH	94 095	84	1 (Reference)		23	1 (Reference)		61	1 (Reference)	
Possible FH	3905	90	27 (20–36)	<0.001	40	43 (26–73)	<0.001	50	20 (14–30)	<0.001
Definite FH	0[a]	—	—	—	0	—	—	0	—	—
Make Early Diagnosis to Prevent Early Death criteria										
Unlikely FH	92 609	134	1 (Reference)		40	1 (Reference)		94	1 (Reference)	
Probable FH	789	40	40 (28–58)	<0.001	23	78 (46–132)	<0.001	17	23 (14–40)	<0.001

[a]A definite diagnosis of familial hypercholesterolaemia requires by the Simon Broome criteria information on mutation carrier status or the presence of tendon xanthoma. We did not have information on tendon xanthoma in the present study, and information on mutation carrier status was on purpose not used in the categorization into diagnostic groups. Low-density lipoprotein cholesterol concentration was multiplied by 1.43 in participants receiving cholesterol-lowering treatment. Participants with hypothyroidism have been excluded ($n = 274$).

clinical diagnostic classification, and this explains the low frequency of carriers in the definite/probable FH categories. LDL-cholesterol concentrations were corrected for use of cholesterol-lowering medication.[22]

Compared with individuals with LDL-cholesterol below 4 mmol/L, odds ratio for carrying an FH mutation was 17 (9–31) for LDL-cholesterol of 4–4.9 mmol/L, 69 (37–126) for LDL-cholesterol of 5–5.9 mmol/L, 132 (66–263) for LDL-cholesterol of 6–6.9 mmol/L, 264 (109–637) for LDL-cholesterol of 7–7.9 mmol/L, and 320 (129–798) for LDL-cholesterol above 7.9 mmol/L (*Figure 1*). Risks of carrying a mutation were more extreme using individuals with LDL-cholesterol below 3 mmol/L as the reference group (see Supplementary material online, *Figure S1*).

To test the ability of LDL-cholesterol concentrations to discriminate between FH mutation carriers and non-carriers, we used receiver operating characteristic curve analysis (*Figure 2*). LDL-cholesterol concentration had in general a high ability to discriminate between FH mutation carriers and non-carriers with an area under the curve of 0.92 (95% CI: 0.90–0.94) for any mutation and 0.92 (0.89–0.95) for an *LDLR* mutation, and had highest predictive ability in those below 40 years of age with an area under the curve of 0.98 (0.97–0.99) for any mutation and 0.99 (0.97–1.00) for an *LDLR* mutation. The most optimal threshold for LDL-cholesterol concentration to discriminate between mutation carriers and non-carriers was 4.4 mmol/L for all ages. The predictive value of LDL-cholesterol was slightly lower in participants on statin treatment: using non-corrected LDL-cholesterol values, the area under the curve was 0.90 (95% CI: 0.88–0.92) for any mutation, and 0.90 (0.87–0.93) for an *LDLR* mutation (see Supplementary material online, *Figure S2*). Also, receiver operating characteristics curves were more attenuated and a specific cut point difficult to identify.

Lipid phenotype of familial hypercholesterolaemia mutation carriers

Among participants not treated with cholesterol-lowering medication and compared with non-carriers, those with an *LDLR* mutation had 2.5 mmol/L (67%) higher LDL-cholesterol, 2.6 mmol/L (37%) higher total cholesterol, and 72 mg/dL (54%) higher apolipoprotein B concentrations (*Figure 3*). The corresponding values for participants with an *APOB* mutation were 1.6 mmol/L (47%) higher LDL-cholesterol, 1.5 mmol/L (25%) higher total cholesterol, and 31 mg/dL (26%) higher apolipoprotein B concentrations. Plasma LDL-cholesterol concentrations were higher and more extreme in *LDLR* mutation carriers compared with *APOB* carriers, and 93% of *LDLR* and 87% of *APOB* carriers off cholesterol-lowering medication had LDL-cholesterol concentrations above the 75th percentile for the general population, although 53% of *LDLR* and 39% of *APOB* carriers off cholesterol-lowering medication had LDL-cholesterol concentrations below the 95th percentile (see Supplementary material online, *Figure S3*).

Risk of coronary artery disease in familial hypercholesterolaemia mutation carriers

On average, carriers of, respectively, an *LDLR* and *APOB* mutation had coronary artery disease 11 and 3 years before and myocardial infarction 13 and 9 years before non-carriers (*Figure 4*). The prevalence of coronary artery disease was 11% in mutation carriers compared with 9% in non-carriers. The multifactorially adjusted odds ratio for coronary artery disease was 3.3 (1.7–6.4) in *LDLR* carriers and 1.3 (0.6–2.5) in *APOB* mutation carriers compared with non-carriers (*Figure 4*). Corresponding odds ratios for myocardial infarction were 5.3 (2.4–12) and 1.8 (0.7–4.6), respectively.

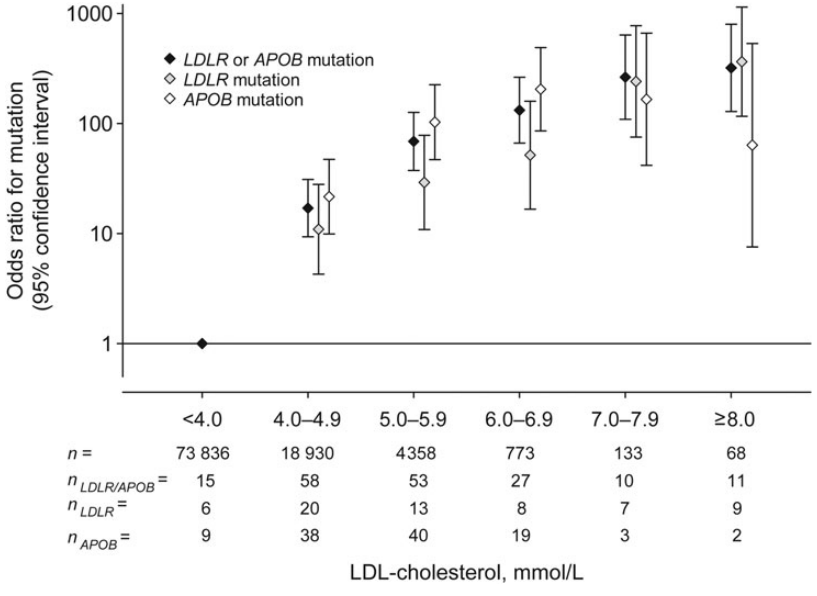

Figure 1 Risk of a low-density lipoprotein receptor (*LDLR*) or an apolipoprotein B (*APOB*) gene mutation as a function of increasing low-density lipoprotein (LDL)-cholesterol levels in the Copenhagen General Population Study. Numbers (*n*) below the figure are number of non-carriers and mutation carriers in each LDL-cholesterol category. To convert cholesterol values to mg/dL, multiply values in mmol/L by 38.6.

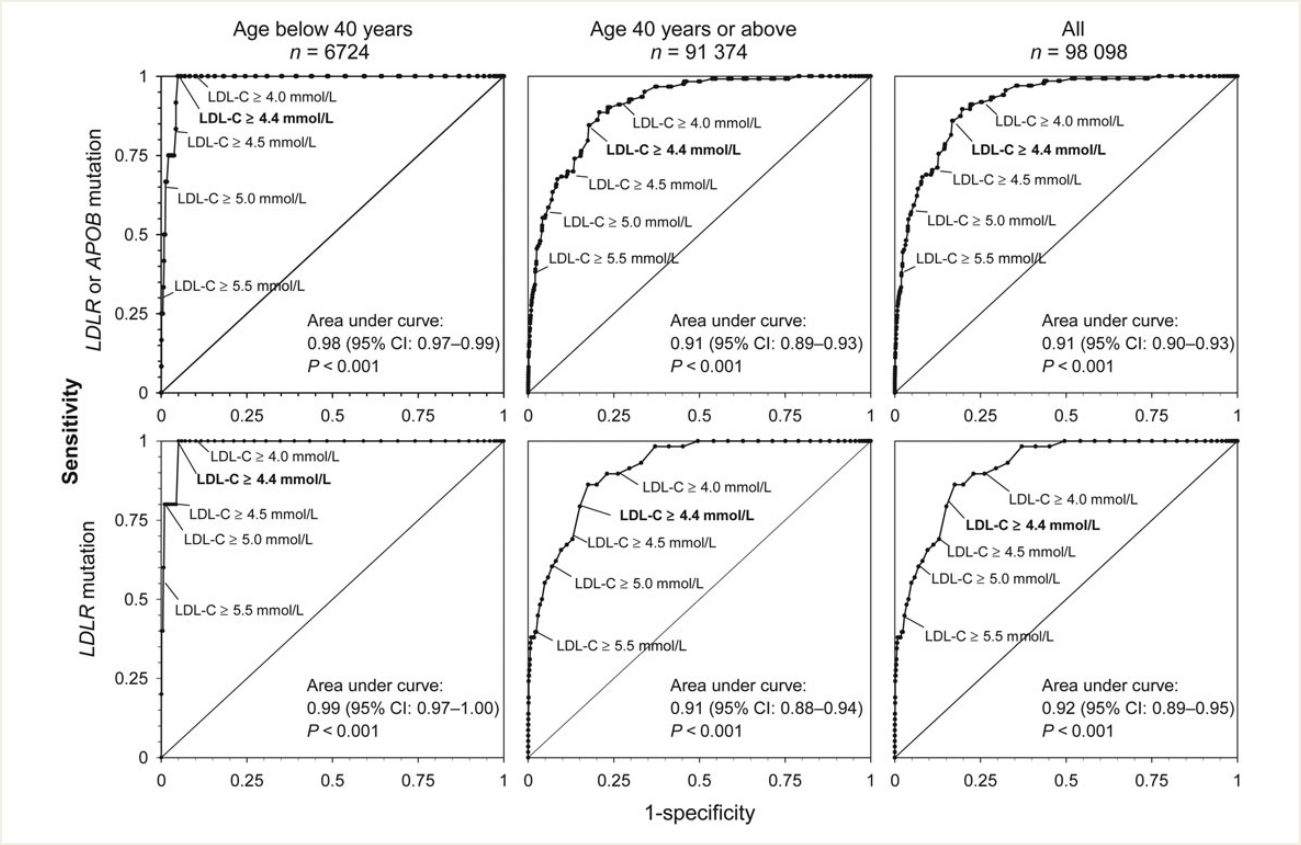

Figure 2 Receiver operating characteristic curves of sensitivity (the true positive rate) as a function of 1-specificity (the false positive rate) for cut points of low-density lipoprotein cholesterol concentrations to discriminate between low-density lipoprotein receptor, *LDLR*, W23X, W66G, or W556S or apolipoprotein B, *APOB*, mutation carriers and non-carriers on upper panels and *LDLR* mutations only below. Each point on the curve represents a sensitivity/1-specificity pair for a specific decision threshold. To convert cholesterol values to mg/dL, multiply values in mmol/L by 38.6. LDL-C, low-density lipoprotein cholesterol.

Discussion

This is the first study ever to report on the prediction of an FH-causing mutation in an unselected general population. The prevalence of *LDLR* [W23X, W66G, W556S] or *APOB* [R3500Q] mutations combined was 0.18% (1:564). Since these mutations account for 38.7% of all known FH-causing mutations tested for in genetic screening services in Copenhagen (Anne Tybjærg-Hansen, from genetic testing of 142 index cases including full sequencing of *LDLR*, *PCSK9* and the receptor-binding region of *APOB* and MLPA analysis of deletions/rearrangements in the *LDLR* gene at Copenhagen University Hospital), this prevalence suggests a total prevalence of known FH mutations of 0.46% (1:217).

The best phenotypic predictors of an FH mutation were a definite and probable diagnosis of FH by the DLCN criteria, and an LDL-cholesterol concentration above 4.4 mmol/L, particularly in individuals aged <40 years. *LDLR* and *APOB* mutation carriers not treated with cholesterol-lowering medication had as previously shown[24] on average 2.5 mmol/L (67%) and 1.6 mmol/L (47%) higher LDL-cholesterol concentrations, respectively, and *LDLR* and *APOB* mutation carriers on average had a myocardial infarction 13 and 9 years before non-carriers.

The prevalence of *LDLR* and *APOB* mutations of 0.18% (1:546) was similar to that previously reported in a smaller Danish community-based study genotyping the same genetic variants.[24,25] As the FH mutations genotyped cover 38.7% of the known FH mutation spectrum in Copenhagen, with a similar proportion in two other parts of Denmark[20,21] (see Supplementary material online, *Table S2*), a cautious estimate of the overall prevalence of disease-causing mutations may be closer to 0.46% (1:217).

This prevalence of FH is comparable to our previous report in a smaller sample of the same population based on phenotypic DLCN criteria alone,[8,26] as well as with the prevalence found in a recent exome sequencing study of US and European individuals.[27] Using an estimated prevalence of known FH mutations of 30 and 50% (rather than 38.7%) in the present study yields an estimate of total prevalence of known FH mutations of 0.59% (1:169) and 0.35% (1:282), respectively.

In the present unselected population, 64% of mutation carriers had an *APOB* mutation and only 36% an *LDLR* mutation. In reports from patient cohorts, *APOB* and *LDLR* mutations are present in, respectively, 4–16% and 84–96% of carriers,[21,28,29] suggesting an ascertainment bias in such studies with a higher referral rate of *LDLR* carriers compared with *APOB* carriers.[24] This may be explained by

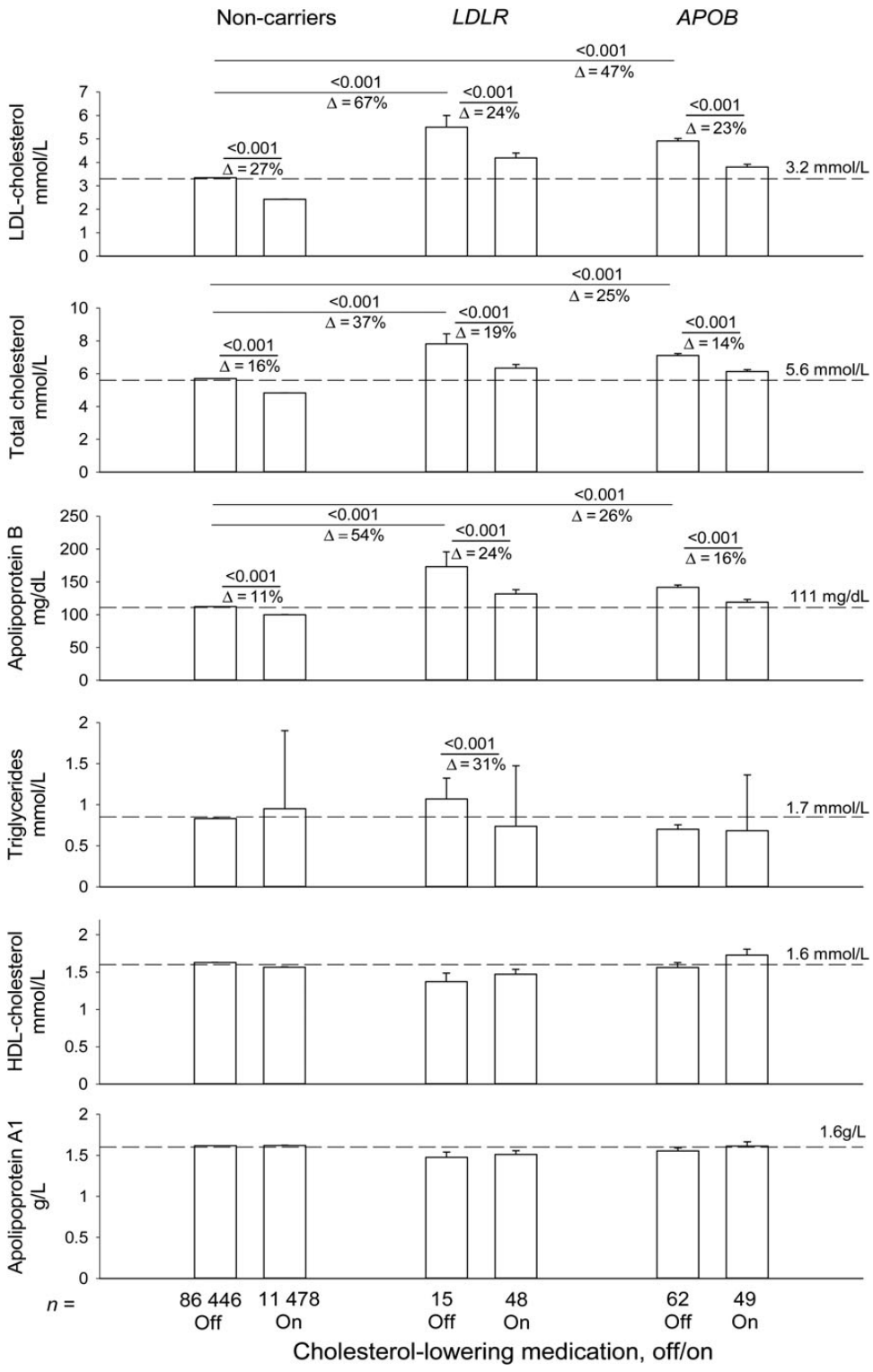

Figure 3 Plasma lipid, lipoprotein, and apolipoprotein concentrations as a function of carrier status of a low-density lipoprotein receptor or an apolipoprotein B gene mutation in individuals from the general population on and off cholesterol-lowering medication. Bars represent mean and whiskers standard error. *P*-values are for comparison between participants off and on cholesterol-lowering treatment, respectively, and between non-carriers and mutation carriers by Student's *t*-test. Vertical broken lines correspond to the mean concentration of the general population. Δ denotes the per cent difference in concentrations between groups. To convert cholesterol values to mg/dL, multiply values in mmol/L by 38.6. To convert triglyceride values to mg/dL, multiply values in mmol/L by 89. *LDLR*, low-density lipoprotein receptor W23X, W66G, or W556S mutation; *APOB*, apolipoprotein B R3500Q mutation; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

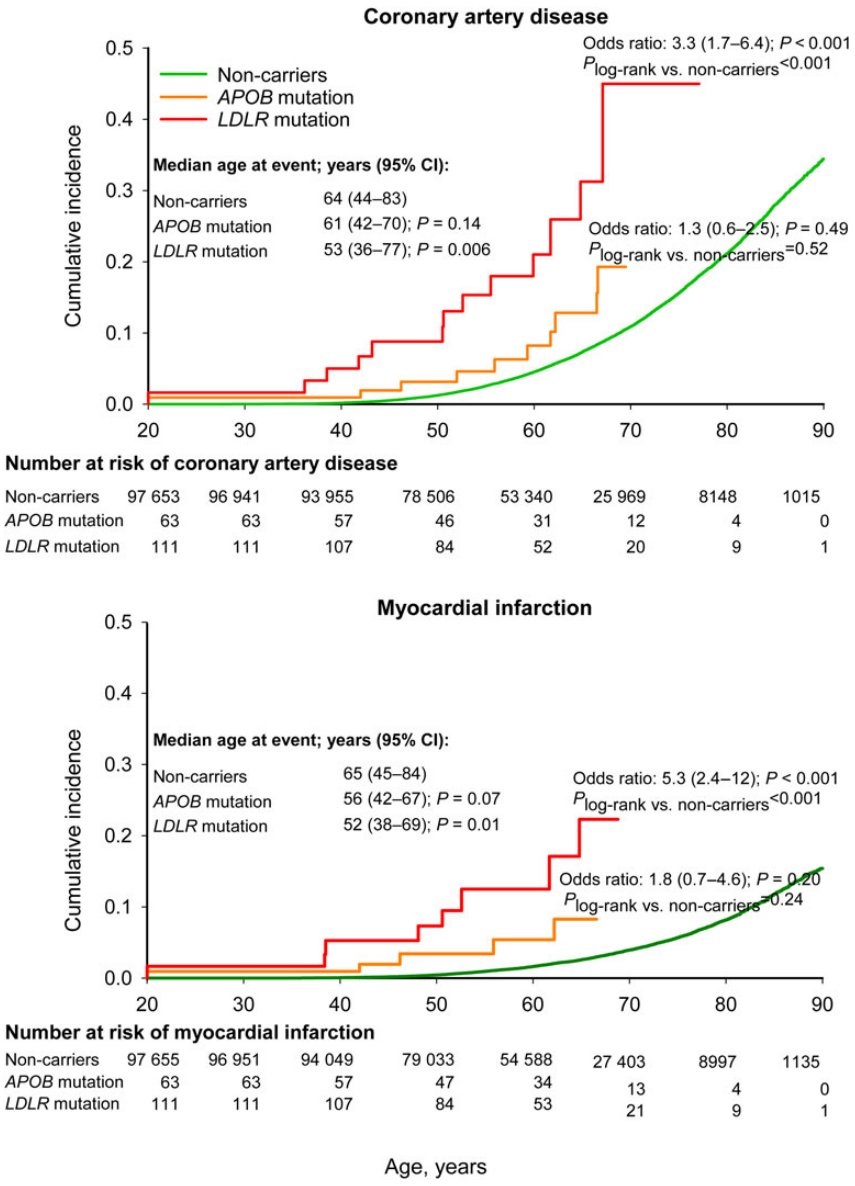

Figure 4 Cumulative incidence and risk of coronary heart disease as a function of mutation carrier status of low-density lipoprotein receptor (*LDLR*) or an apolipoprotein B (*APOB*) gene mutations and age. Median age at event with 95% confidence interval (CI) is shown on each panel and number of persons at risk at different age points below panels. Log-rank *P*-values and odds ratios with 95% confidence interval are for differences in risk between mutation carriers and non-carriers by log-rank test and logistic regression.

the more severe phenotype in *LDLR* carriers with higher LDL-cholesterol concentrations and perhaps also a higher frequency of the characteristic tendon xanthomas or may be due to a founder mutation effect in the Danish population. Also, the occurrence of an *APOB* mutation in 64% of the four mutations screened for in the general population may suggest that the four mutations tested in the present population may account for >38.7% of the mutation population: if one assumes that the high prevalence of *APOB* mutations in the screened population means that the four mutations screened for accounts for >38.7%, say 40.0%, the prevalence of FH in the population will be lower, i.e. 1:222, instead of 1:217. Furthermore; *APOB* mutations comprise 64% of the four mutations screened in the general population, but in the data from genetic screening services in Copenhagen (Anne Tybjærg-Hansen, from genetic testing of 142 index cases including full sequencing of *LDLR*, *PCSK9* and the receptor-binding region of *APOB* and MLPA analysis of deletions/rearrangements in the *LDLR* gene at Copenhagen University Hospital), other *LDLR* mutations, not tested in the general population, occur in 61% (51 and 5%, see Supplementary material online, *Table S2*), so when comparing the results to other cohorts, the *APOB* mutation percentage of the total number of mutations must be used, and this percentage is considerably <64%.

Also, the occurrence of an *APOB* mutation in 64% of mutation carriers in the general population may suggest that the four mutations tested for in the present population may account for >38.7% of the mutation population, and the 'true' prevalence could be higher than the estimated 0.46% (1:217).

Previous studies on prevalence of *LDLR* and *APOB* mutations among the clinical diagnostic groups according to the three commonly used diagnostic criteria have been conducted retrospectively in patients from lipid clinics. Mutation rates of 63–80% have been reported among patients classified with definite FH according to the DLCN criteria;[11,21,30,31] of 32–61% among patients with definite FH according to the Simon Broome criteria;[21,31,32] and 52–83% among patients with FH according to the MEDPED criteria.[21,31] In the present study of a community-based population, the corresponding mutation rates were for the diagnostic categories 24, 2.3, and 5%, respectively, and 0.07, 0.09, and 0.14% among those categorized as unlikely FH. These results may to some extent be due to ascertainment bias, given the more severe phenotype of patients referred to lipid clinics compared with FH in a general population,[24] but nevertheless support using the DLCN phenotypic criteria for predicting the presence of a mutation. In our study, information on mutation was evidently excluded from the classification of FH, obviating a definite FH diagnosis by the Simon Broome criteria and minimizing a definite FH diagnosis by the DLCN criteria. This simulates clinic practice that is usually based on phenotypic findings alone and underscores the usefulness of genetic testing in identifying FH, even in a population with a low probability of mutations.[21] It is noteworthy that numerically most of the FH patients were found in the phenotypically 'possible' and 'unlikely' categories. This may be an argument for general screening for FH at a young age and also for wider use of genetic testing in adult populations.[21,33] Studies of phenotypic predictors of FH mutations among FH patients have been disappointing. One study has concluded that the presence of tendon xanthomas in the patient or a relative and an LDL-cholesterol concentration above 4.9 mmol/L is the best predictor of mutation in a clinic-based sample.[31] The association between the phenotype and genotype can be complex in FH. This could be because only a proportion of FH cases are caused by Mendelian inheritance of mutations in known *LDLR*, *APOB,* and *PCSK9* genes, while others are explained by either unknown single genes or polygenic inheritance,[3,21] or the phenotype may be modified by other genetic variants.[34]

In the present study, the best phenotypic predictors of an FH mutation among those included in the diagnostic criteria were a definite and a probable FH diagnosis by the DLCN criteria. The DLCN criteria combine information from the best single criteria identified in the present study, that is, a high LDL-cholesterol concentration, a family history of hypercholesterolemia, and premature coronary artery disease into a score, weighting the contribution of each criterion to the total diagnosis, and it seems this combined score is better than the single criteria. As all the three established diagnostic criteria and two recently suggested ascertainment flows (FAMCAT[9] and the Canadian Cardiovascular Society Position Statement on Familial Hypercholesterolemia[35]) rely on LDL-cholesterol concentrations, we attempted to identify the ideal cut point for LDL-cholesterol concentration using receiver operating characteristic statistics and found that a cut point of 4.4 mmol/L resulted in a sensitivity of 99.5% and a specificity of 97%. This sensitivity and specificity are promising and comparable to those observed in a study of children;[36] however, owing to the low *a priori* probability of carrying a mutation in a community-based population compared with patients attending lipid clinics, the positive predictive value is low. The impact of the *a priori* probability of an FH mutation was shown in an early study observing that using a total cholesterol level of 8.0 mmol/L or higher as a diagnostic cut point, 4% of persons in the general population would have clinical FH, but 95% of persons who were first-degree relatives of known FH mutation carriers cases would have FH.[18] These observations lead to the first MEDPED criteria.[18] However, this scenario with a low *a priori* probability of mutation is the challenge that the clinicians face identifying the patients at the highest risk of carrying a mutation to refer for genetic testing. This is also seen in the FAMCAT where an LDL-cholesterol(>4.9 mmol/L) or total cholesterol(>7.5 mmol/L) used to identify clinical FH patients performed relatively poorly because of a low prevalence of clinical FH of 1:589 in the population.[9] The Canadian Cardiovascular Society Position Statement on Familial Hypercholesterolemia suggests a diagnostic flow first including individuals with LDL-cholesterol >5 mmol/L, secondly ruling out individuals with secondary causes of dyslipidaemia, and finally diagnosing FH in those with a first-degree relative with LDL-cholesterol >5 mmol/L or early coronary heart disease or physical signs of FH.[35] A cut point of >5 mmol/L would in our population result in a specificity >98%, but a sensitivity between 65 and 80%, thus in the first diagnostic step potentially miss individuals with FH.

As shown previously,[24] in the present study, *LDLR* and *APOB* mutation carriers not treated with cholesterol-lowering medication had on average 2.5 mmol/L (67%) and 1.6 mmol/L (47%) higher LDL-cholesterol compared with non-carriers in the general population. Only 76% of *LDLR* and 44% of *APOB* mutation carriers were treated with cholesterol-lowering medication. *LDLR* and *APOB* mutation carriers treated with cholesterol-lowering medication had mean LDL-cholesterol concentrations of 3.8 and 4.2 mmol/L, respectively, indicating that the recommended treatment targets of LDL-cholesterol of <2.5 mmol/L in adults and <1.8 in adults with coronary heart disease or diabetes[3] are not reached.

Early association studies reported increased risk of coronary artery disease among clinical FH patients compared with non-diseased family members.[7,37] Later studies have unequivocally shown that FH mutation carriers have high risk of coronary artery disease, although these results are from selected patient populations. One study of FH patients observed an odds ratio for coronary artery disease of 1.8 (1.1–3.1) in *LDLR* carriers, 3.4 (0.7–16) in *APOB* carriers, and 20 (1.9–211) in *PCSK9* carriers vs. non-carriers.[28] Another study of 9 912 *LDLR* mutation carriers referred for genetic testing compared with 18 393 non-carrier relatives reported a hazard ratio of 3.6 (3.2–4.1).[38] In the present study of a community-based population, the adjusted odds ratio for coronary artery disease was 11 (5.0–25) in *LDLR* carriers and 4.7 (1.7–14) in *APOB* carriers on cholesterol-lowering medication compared with non-carriers off medication. The high risk of coronary artery disease observed in both mutation carriers and non-carriers on cholesterol-lowering medication may be explained by the cross-sectional design, noting that those diagnosed with coronary artery disease are likely to be receiving statin

treatment or by selection of persons with multiple cardiovascular risk factors who would also be on statin treatment; indeed, adjustment for age, gender, body mass index, metabolic syndrome, diabetes mellitus, hypertension, and smoking accentuated risk estimates (data not shown). Also, in the present study, *LDLR* and *APOB* mutation carriers on average had myocardial infarction 13 and 9 years earlier compared with non-carriers, supporting that carrying an FH mutation is in itself a risk factor for coronary artery disease, and that early screening and treatment may reduce risk.

Limitations of the present study are as follows: (i) Information on tendon xanthomas was not available, so this criterion is not evaluated with regard to predictive value and could also not contribute to the diagnosis of FH using the DLCN and Simon Broome criteria. This might have resulted in a slight underestimation of the prevalence of FH. Specifically, the presence of tendon xanthomas and corneal arcus carry great weight in all three diagnostic tools and failure to consider them may alter the enrichment of FH mutation carriers among those classified as definite or probable FH; (ii) We only screened for three known *LDLR* and one *APOB* mutations accounting for an estimated 38.7% of FH-causing mutations in Copenhagen FH patients. Only four mutations within two FH genes were used as a standard for extrapolating total prevalence, while mutations within other known FH genes were omitted; however, mutations in other FH genes are much rarer than those in *LDLR* and *APOB*,[3–5] also illustrated by the fact that no *PCSK9* mutations were found in FH genetic screening services at Copenhagen University Hospital (see Supplementary material online, *Table S2*). Therefore, the sensitivity and specificity measures provided only reflect a partial screen for FH-causing mutations. This may cause an underestimation of the prevalence of FH-causing mutations in the general population, and may, as the mutations to some degree are founder mutations, limit the applicability of the results to other populations with a very different mutation spectrum. (iii) We did not have baseline plasma LDL-cholesterol concentrations measured off cholesterol-lowering medication, but corrected the on-treatment LDL-cholesterol values by multiplying with 1.43,[22] corresponding to an estimated 30% reduction in LDL-cholesterol with standard cholesterol-lowering medication. This is an assumption and may have caused some misclassification of participants; (iv) LDL-cholesterol in children and family and personal details of tendon xanthoma or corneal arcus were not recorded in our study, potentially resulting in an underestimation of the predictive value of the combined diagnostic criteria; (v) Risk estimates for coronary artery disease in mutation carriers on lipid-lowering treatment may be prone to selection bias as treatment may have been prescribed for the presence of disease; (vi) Also, the study only included participants between ages 20 and 100 years, and results may not be applicable to children.

Known FH-causing mutations are estimated to occur in 1:217 in the general population in Copenhagen and are best identified by a definite or probable phenotypic diagnosis of FH based on the DLCN criteria, or an LDL-cholesterol above 4.4 mmol/L and particularly in individuals below 40 years of age. Genetic screening facilitates diagnosis and risk assessment of FH; however, one must treat the phenotype not the genotype, and LDL-cholesterol should be lowered as early as possible to recommended levels regardless of information on mutation.[3,5]

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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Conflict of interest: M.B. and B.G.N. performed statistical analysis; M.B. and B.G.N. acquired the data; M.B., G.F.W., A.T.-H., and B.G.N. conceived and designed the research; M.B. drafted the manuscript; M.B., G.F.W., A.T.-H., and B.G.N. made critical revision of the manuscript for key intellectual content.

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