High lipoprotein(a) as a possible cause of clinical familial hypercholesterolaemia: a prospective cohort study

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Summary

Background The reason why lipoprotein(a) concentrations are raised in individuals with clinical familial hypercholesterolaemia is unclear. We tested the hypotheses that high lipoprotein(a) cholesterol and *LPA* risk genotypes are a possible cause of clinical familial hypercholesterolaemia, and that individuals with both high lipoprotein(a) concentrations and clinical familial hypercholesterolaemia have the highest risk of myocardial infarction.

Methods We did a prospective cohort study that included data from 46 200 individuals from the Copenhagen General Population Study who had lipoprotein(a) measurements and were genotyped for common familial hypercholesterolaemia mutations. Individuals receiving cholesterol-lowering drugs had their concentrations of LDL and total cholesterol multiplied by 1·43, corresponding to an estimated 30% reduction in LDL cholesterol from the treatment. In lipoprotein(a) cholesterol-adjusted analyses, total cholesterol and LDL cholesterol were adjusted for the lipoprotein(a) cholesterol content by subtracting 30% of the individuals' lipoprotein(a) total mass before total and LDL cholesterol were used for diagnosis of clinical familial hypercholesterolaemia. We used modified Dutch Lipid Clinic Network (DLCN), Simon Broome, and Make Early Diagnosis to Prevent Early Death (MEDPED) criteria to clinically diagnose familial hypercholesterolaemia. Cox proportional hazard regression calculated hazard ratios (95% CI) of myocardial infarction.

Findings Using unadjusted LDL cholesterol, mean lipoprotein(a) concentrations were 23 mg/dL in individuals unlikely to have familial hypercholesterolaemia, 32 mg/dL in those with possible familial hypercholesterolaemia, and 35 mg/dL in those with probable or definite familial hypercholesterolaemia (p_{trend}<0·0001). However, when adjusting LDL cholesterol for lipoprotein(a) cholesterol content the corresponding values were 24 mg/dL for individuals unlikely to have familial hypercholesterolaemia, 22 mg/dL for those with possible familial hypercholesterolaemia, and 21 mg/dL for those with probable or definite familial hypercholesterolaemia (p_{trend}=0·46). High lipoprotein(a) cholesterol accounted for a quarter of all individuals diagnosed with clinical familial hypercholesterolaemia and *LPA* risk genotypes were more frequent in clinical familial hypercholesterolaemia, whereas lipoprotein(a) concentrations were similar in those with and without familial hypercholesterolaemia mutations. The hazard ratios (HRs) for myocardial infarction compared with individuals unlikely to have familial hypercholesterolaemia and lipoprotein(a) concentration of 50 mg/dL or less were 1·4 (95% CI 1·1–1·7) in those unlikely to have familial hypercholesterolaemia and lipoprotein(a) concentrations of more than 50 mg/dL, 3·2 (2·5–4·1) in those with possible, probable, or definite familial hypercholesterolaemia and lipoprotein(a) concentration of 50 mg/dL or less, and 5·3 (3·6–7·6) in those with possible, probable, or definite familial hypercholesterolaemia and lipoprotein(a) concentration of more than 50 mg/dL. In analyses using Simon Broome or MEDPED criteria, results were similar to those using DLCN criteria to diagnose clinical familial hypercholesterolaemia.

Interpretation High lipoprotein(a) concentrations and corresponding *LPA* risk genotypes represent novel risk factors for clinical familial hypercholesterolaemia. Our findings suggest that all individuals with familial hypercholesterolaemia should have their lipoprotein(a) measured in order to identify those with the highest concentrations, and as a result, the highest risk of myocardial infarction.

Funding Danish Heart Association and IMK General Fund, Denmark.

Lancet Diabetes Endocrinol 2016

Published Online
May 12, 2016
http://dx.doi.org/10.1016/S2213-8587(16)30042-0

See Online/Comment
http://dx.doi.org/10.1016/S2213-8587(16)30082-1

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Introduction

High lipoprotein(a) concentration is a common causal genetic risk factor for myocardial infarction.[1–5] Lipoprotein(a) consists of an LDL particle containing cholesterol and apolipoprotein B100 bound to apolipoprotein(a) (which is encoded by the *LPA* gene).[5] Lipoprotein(a) concentration is largely genetically determined; the most important genetic determinants in *LPA* for lipoprotein(a) concentrations are the kringle IV type 2 (KIV-2) size polymorphisms, which are inversely associated with lipoprotein(a) concentrations, and the rs10455872 single nucleotide polymorphism. The inverse association with KIV-2 is because the large isoforms are captured and degraded intracellularly in the liver, whereas the small isoforms remain in the circulation.[5]

Familial hypercholesterolaemia is a common genetic cause of premature myocardial infarction due to lifelong increased LDL cholesterol concentrations.[6,7] There are many known causal mutations for familial hypercholesterolaemia, with more than 95% identified in the LDL receptor gene (*LDLR*), 2–11% in the apolipoprotein B

Research in context

Evidence before this study

We searched PubMed and Web of Science using the search terms "lipoprotein a", "lpa", "familial hypercholesterolemia", and "FH" for articles published up to Jan 3, 2016. High lipoprotein(a) concentrations have been associated with myocardial infarction, and lipoprotein(a) concentration is largely genetically determined. Familial hypercholesterolaemia is a common genetic cause of myocardial infarction, due to lifelong elevated LDL cholesterol levels. Many known familial hypercholesterolaemia-causing mutations exist, with more than 95% identified in the LDL receptor, *LDLR*, 2–11% in apolipoprotein B, *APOB*, and less than 1% in proprotein convertase subtilisin/kexin type 9, *PCSK9*. The role of lipoprotein(a) and the *LPA* gene in familial hypercholesterolaemia is still unclear.

Added value of this study

Our results from 46 200 individuals from the Copenhagen General Population Study who had lipoprotein(a) measurements and were genotyped for common familial hypercholesterolaemia mutations show that a 39–58% increased lipoprotein(a) concentration in individuals with clinical familial hypercholesterolaemia was explained by lipoprotein(a) cholesterol contributing to LDL cholesterol in the clinical diagnosis of familial hypercholesterolaemia. Among all individuals with clinical familial hypercholesterolaemia, a quarter obtained the diagnosis because of high lipoprotein(a) concentrations, and high lipoprotein(a) concentrations contributed to an increased risk of myocardial infarction on top of the already high risk caused by high LDL cholesterol in patients with clinical familial hypercholesterolaemia.

Implications of all the available evidence

These novel findings suggest that the *LPA* gene coding for apolipoprotein(a) in lipoprotein(a), and thus determining plasma lipoprotein(a) concentrations, is possibly the second most frequent cause of clinical familial hypercholesterolaemia. These data suggest that all individuals with familial hypercholesterolaemia should have lipoprotein(a) concentrations measured to identify those with the highest concentrations and, as a result, the highest risk of myocardial infarction.

gene (*APOB*), and less than 1% in the proprotein convertase subtilisin/kexin type 9 gene (*PCSK9*). These mutations lead to decreased clearance of LDL cholesterol from plasma via the LDL receptor, and consequently to increased LDL cholesterol concentrations. Individuals with clinically diagnosed homozygous and heterozygous familial hypercholesterolaemia also have increased lipoprotein(a) concentrations,[6–10] but the reason for this finding is unclear. It could either be because reduced LDL receptor function leads to high lipoprotein(a) concentration via decreased clearance of lipoprotein(a) from plasma or because high lipoprotein(a) cholesterol is a cause of clinical familial hypercholesterolaemia. Although levels of circulating lipoprotein(a) might be mostly determined by its rate of synthesis, clearance through the LDL receptor seems to play at least some part, as exemplified by the fairly robust 25–30% reduction in lipoprotein(a) caused by PCSK9 inhibitors, including in patients with heterozygous familial hypercholesterolaemia.[11,12]

See Online for appendix

In this study, we tested the hypotheses that high lipoprotein(a) cholesterol and *LPA* risk genotypes are a possible cause of clinical familial hypercholesterolaemia, and that individuals with both high lipoprotein(a) concentrations and clinical familial hypercholesterolaemia have the highest risk of myocardial infarction.

Methods

Study design and participants

This study is based on data from the Copenhagen General Population Study biobank cohort. In this prospective cohort study, adults (aged 20–100 years) of Danish descent were selected at random from the national Danish Civil Registration System (n=3 844 883) between 2003 and 2014 to reflect the adult, white population of Copenhagen (roughly 10 000 individuals examined yearly, with a 43% response rate). Among 99 441 participants, we included the 46 200 consecutive individuals with a lipoprotein(a) measurement; masked to all other data in the study (see appendix p 2) for characteristics of included and excluded participants); of these individuals, 38 555 also had *LPA* KIV-2 repeat numbers measured. All participants completed a self-administered questionnaire (which was checked by an examiner on the day of study attendance), underwent a physical examination, and gave blood for biochemical analyses and DNA testing, all during a single hospital visit. The present study was approved by the ethical committee for the Capital Region of Denmark and by Herlev and Gentofte Hospital, and written informed consent was obtained from all participants.

Procedures

Total cholesterol, HDL cholesterol, triglycerides, and glucose concentrations were measured with standard hospital assays. LDL cholesterol was measured directly with a homogeneous assay from Thermo Fisher (Thermo Fisher Scientific, Vantaa, Finland) when triglycerides were more than 4 mmol/L, and otherwise calculated by use of the Friedewald formula. Turbidimetric apolipoprotein(a) isoform-insensitive assays were used to measure concentrations of lipoprotein(a) total mass.[13] 46 200 individuals had measurements of lipoprotein(a); the remaining 53 241 individuals did not have lipoprotein(a) measurements because at the time of

recruitment, no optimum isoform-insensitive assay was available. Individuals receiving cholesterol-lowering drugs had their concentrations of LDL and total cholesterol multiplied by 1·43, corresponding to an estimated 30% reduction in LDL cholesterol from the treatment.[14] In lipoprotein(a) cholesterol-adjusted analyses, total cholesterol and LDL cholesterol were adjusted for the lipoprotein(a) cholesterol content by subtracting 30% of the individuals' lipoprotein(a) total mass[15] before total and LDL cholesterol were used for diagnosis of clinical familial hypercholesterolaemia. Kinpara and colleagues[15] reported that the mean cholesterol content of total lipoprotein(a) mass was roughly 30% and that lipoprotein(a) cholesterol is co-measured in total and LDL cholesterol measurements;[15] however, this proportion might be an underestimate so we also included an analysis subtracting LDL cholesterol for 45% of lipoprotein(a) total mass.[5] The correlation between unadjusted LDL cholesterol and LDL cholesterol adjusted for lipoprotein(a) content is shown in the appendix (p 6). The appendix (pp 17–18) provides more information about lipoprotein(a) measurements, genotyping, and other covariates, such as hypertension, diabetes, family history of cardiovascular disease, and peripheral vascular disease for adjustment.

We used the three most common standardised criteria to clinically diagnose familial hypercholesterolaemia: the Dutch Lipid Clinic Network (DLCN) criteria, the Simon Broome criteria, and the Make Early Diagnosis to Prevent Early Death (MEDPED) criteria (appendix p 3).[16] We modified these criteria because information about LDL cholesterol in children of participants as well as family and personal details of tendon xanthoma or corneal arcus were not recorded in our study. To mimic the information available at an individual's first visit to the doctor, information about familial hypercholesterolaemia-related mutations was not taken into account for the diagnosis of clinical familial hypercholesterolaemia.

Using the DLCN criteria,[17] we regarded a diagnosis of clinical familial hypercholesterolaemia as definite if the total score was greater than 8, probable if the score was 6–8, possible if the score was 3–5, and unlikely if the score was below 3 points (appendix p 3). The score was calculated with points assigned for presence of family history of a first-degree relative with known premature (age <55 years for men and <60 years for women) coronary artery disease or vascular disease, or a first-degree relative with known hypercholesterolaemia, or both (1 point); presence of personal history of premature coronary artery disease (2 points) or premature cerebral or peripheral vascular disease (1 point, if not already 2 points for premature coronary artery disease); or LDL cholesterol concentration greater than 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), or 4·0–4·9 mmol/L (155–189 mg/dL; 1 point; only the highest LDL criteria generate points).

Using the Simon Broome criteria,[18] a diagnosis of clinical familial hypercholesterolaemia was deemed possible if total cholesterol concentration was more than 7·5 mmol/L (290 mg/dL) or LDL cholesterol concentration was more than 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 (appendix p 3). Otherwise, individuals were classified as unlikely to have familial hypercholesterolaemia.

Using the MEDPED criteria,[19] a diagnosis of probable familial hypercholesterolaemia was considered for people aged 20–29 years if total cholesterol was 7·5 mmol/L (290 mg/dL) or more, or LDL cholesterol concentration was 5·7 mmol/L (220 mg/dL) or more; for people aged 30–39 years if total cholesterol was 8·8 mmol/L (340 mg/dL) or more, or LDL cholesterol concentration was 6·2 mmol/L (240 mg/dL) or more; and for people aged 40 years or more if total cholesterol was 9·3 mmol/L (360 mg/dL) or more, or LDL cholesterol concentration was 6·7 mmol/L (260 mg/dL) or more (appendix p 3). Otherwise, individuals were classified as unlikely to have familial hypercholesterolaemia.

Statistical analysis

We used χ^2 analysis to test for departures from Hardy-Weinberg equilibrium of distribution of genotypes, and for distribution of dichotomous variables between categories of clinical familial hypercholesterolaemia. We used Cuzick's non-parametric test for trend or the Mann-Whitney test to assess differences in continuous variables between different categories of clinical familial hypercholesterolaemia and between familial hypercholesterolaemia mutation carriers and non-carriers.

For prospective analyses on risk of myocardial infarction, we compared individuals stratified for clinical familial hypercholesterolaemia and lipoprotein(a) concentrations or *LPA* KIV-2 repeat number compared with individuals without familial hypercholesterolaemia and low lipoprotein(a) concentrations or high KIV-2 repeat number. For lipoprotein(a), we chose a cutoff point of 50 mg/dL (roughly the 80th percentile), as recommended by the European Atherosclerosis Society Consensus Panel,[4] and likewise we chose the bottom 20th percentile for KIV-2 repeat number, because these two measurements have an inverse association. We analysed age at event using left truncation (delayed entry) and age as time-scale. Thus, age was automatically adjusted for, and the fact that the period of ignorance (their whole life until entering the study) exists before an individual enters the study during which the individual has been subjected to the effects of high lipoprotein(a) concentrations was taken into account. Proportionality of hazards over time was assessed by plotting –ln(–ln[survival]) versus ln(analysis time). No major violations of the proportional-hazard assumption were

identified. We used Cox proportional-hazards regression to calculate hazard ratios (HRs) with 95% CIs, adjusted multifactorially for sex, age (as timescale), smoking status, hypertension, diabetes, and use of cholesterol-lowering drugs. Individuals who had a recorded myocardial infarction before entry into the study were excluded from the analyses. Cumulative incidences of myocardial infarction were plotted, and differences between groups were examined with log-rank trend tests. We used Stata Statistical Software (release 13) for all statistical analyses.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or the writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

The table shows the baseline characteristics of individuals included in the study. When we clinically diagnosed familial hypercholesterolaemia using the modified DLCN criteria without taking familial hypercholesterolaemia mutations into account, 37 individuals had definite familial hypercholesterolaemia, 147 had probable familial hypercholesterolaemia, 3082 had possible familial hypercholesterolaemia, and 42 934 were unlikely to have familial hypercholesterolaemia. 76 individuals had a familial hypercholesterolaemia-causing mutation—*LDLR* Trp23X (n=3), *LDLR* Trp66Gly (n=22), *LDLR* Trp556Ser (n=2), and *APOB* Arg3500Gln (n=49)—resulting in a combined prevalence of these familial hypercholesterolaemia mutations of 0·16%. These four familial hypercholesterolaemia mutations are all common in Denmark,[16] but only represent 38·7%[20] of all identified familial hypercholesterolaemia mutations. No genotypes deviated from the Hardy-Weinberg expectations (p>0·82 for all). Baseline characteristics by mutation status are shown in the appendix (p 4). During 246 326 person-years of follow-up, 770 events of myocardial infarction were recorded, with a median follow-up time of 3·9 years (IQR 2·9–5·0).

Using DLCN criteria and unadjusted LDL cholesterol to clinically diagnose familial hypercholesterolaemia, mean lipoprotein(a) concentrations were 23 mg/dL (95% CI

	Unlikely to have familial hypercholesterolaemia (n=42 934)	Possible familial hypercholesterolaemia (n=3082)	Probable or definite familial hypercholesterolaemia (n=184)	p_{trend}
Using unadjusted LDL cholesterol to clinically diagnose familial hypercholesterolaemia				
Women	23 234 (54%)	1675 (54%)	113 (62%)	0·31
Age (years)	59 (49–68)	59 (52–67)	58 (51-66)	<0·0001
Total cholesterol (mmol/L)	5·5 (4·8–6·2)	7·4 (6·3–7·9)	8·1 (7·2-9·2)	<0·0001
LDL cholesterol (mmol/L)	3·1 (2·5–3·7)	5·1 (3·9–5·4)	5·6 (4·8-6·7)	<0·0001
Hypertension	26 160 (61%)	2234 (73%)	138 (75%)	<0·0001
Current smokers	7623 (18%)	751 (24%)	52 (28%)	<0·0001
Diabetes	2338 (5%)	211 (7%)	13 (7%)	0·0008
Receiving cholesterol-lowering drugs	4801 (11%)	727 (24%)	96 (52%)	<0·0001
Family history of premature cardiovascular disease or hypercholesterolaemia	14 175 (33%)	1442 (47%)	139 (76%)	<0·0001
Premature peripheral vascular disease	532 (1%)	145 (5%)	7 (4%)	<0·0001
Using LDL cholesterol adjusted for lipoprotein(a) cholesterol to clinically diagnose familial hypercholesterolaemia				
n	43 699	2360	141	
Women	23 731 (54%)	1222 (54%)	69 (60%)	0·81
Age (years)	59 (49–68)	59 (52–66)	59 (52–66)	0·03
Total cholesterol (mmol/L)	5·5 (4·9–6·2)	7·5 (6·8–8·1)	9·6 (8·5–10)	<0·0001
LDL cholesterol (mmol/L)	3·1 (2·5–3·7)	5·1 (4·4–5·5)	6·8 (6·5–7·5)	<0·0001
Hypertension	26 785 (61%)	1656 (73%)	95 (76%)	<0·0001
Current smokers	7827 (18%)	568 (25%)	33 (26%)	<0·0001
Diabetes	2395 (5%)	171 (8%)	8 (7%)	<0·0001
Receiving cholesterol-lowering drugs	4988 (11%)	566 (25%)	70 (57%)	<0·0001
Family history of premature cardiovascular disease or hypercholesterolaemia	14 487 (33%)	1162 (51%)	107 (91%)	<0·0001
Premature peripheral vascular disease	573 (1%)	105 (5%)	6 (5%)	<0·0001

Data are n (%) or median (IQR) . Data are based on 46 200 white individuals from the Copenhagen General Population Study with lipoprotein(a) measurements. Total and LDL cholesterol concentrations were multiplied by 1·43 if individuals were receiving cholesterol-lowering drugs. DLCN=Dutch Lipid Clinic Network.

***Table*: Baseline characteristics, by clinical diagnosis of familial hypercholesterolaemia (modified DLCN criteria)**

22·8–23·3) in individuals categorised as unlikely to have familial hypercholesterolaemia, 32 mg/dL (31–34) in those with possible familial hypercholesterolaemia (ie, 39% higher than those unlikely to have familial hypercholesterolaemia), and 35 mg/dL (29–41) in those with probable or definite familial hypercholesterolaemia (52% higher than in those unlikely to have familial hypercholesterolaemia; $p_{trend}<0·0001$; figure 1). However, when adjusting the LDL cholesterol for lipoprotein(a) cholesterol content, the corresponding values were 24 mg/dL (23·5–24·1) in individuals unlikely to have familial hypercholesterolaemia, 22 mg/dL (21–24) in those with possible familial hypercholesterolaemia, and 21 mg/dL (16–26) in those with probable or definite familial hypercholesterolaemia ($p_{trend}=0·46$; figure 1). Also, using the Simon Broome criteria and unadjusted total and LDL cholesterol, lipoprotein(a) concentrations were 23 mg/dL (22·9–23·5) in individuals unlikely to have familial hypercholesterolaemia and 36 mg/dL (34–38; ie, 57% higher) in those with possible familial hypercholesterolaemia (p<0·0001); corresponding values using total and LDL cholesterol adjusted for lipoprotein(a) cholesterol content were 24 mg/dL (23–24) in individuals unlikely to have familial hypercholesterolaemia and 24 mg/dL (22–36) in those with possible familial hypercholesterolaemia (p=0·26). Finally, using the MEDPED criteria and unadjusted total and LDL cholesterol, lipoprotein(a) concentrations were 24 mg/dL (23–24) in individuals unlikely to have familial hypercholesterolaemia and 38 mg/dL (33–43; ie, 58% higher) in those with probable familial hypercholesterolaemia (p<0·0001); corresponding values using total and LDL cholesterol adjusted for lipoprotein(a) cholesterol content were 24 mg/dL (23–24) in individuals unlikely to have familial hypercholesterolaemia and 23 mg/dL (20–27) in those with probable familial hypercholesterolaemia (p=0·74). When we adjusted the LDL cholesterol for 45% lipoprotein(a) cholesterol content, the results were similar (appendix p 7). Furthermore, median concentrations of lipoprotein(a) by clinical familial hypercholesterolaemia status are shown in the appendix (p 8). Overlap of individuals with clinical familial hypercholesterolaemia by DLCN, Simon Broome, and MEDPED criteria and familial hypercholesterolaemia mutations is shown in the appendix (p 9) and the distribution of lipoprotein(a) concentrations in participants with and without familial hypercholesterolaemia according to the DLCN criteria are also shown in the appendix (p 10).

For clinical diagnosis of familial hypercholesterolaemia based on DLCN criteria and excluding lipoprotein(a) cholesterol content in total and LDL cholesterol values, 43 (23%) fewer individuals had probable or definite familial hypercholesterolaemia and 722 (23%) fewer individuals had possible familial hypercholesterolaemia than when total and LDL cholesterol values were unadjusted (figure 1). Correspondingly, with the Simon Broome criteria, 452 (24%) fewer individuals had possible familial hypercholesterolaemia, and with the MEDPED criteria, 87 (24%) fewer individuals had probable familial hypercholesterolaemia (figure 1).

In accordance with this finding, when DLCN criteria were used to clinically diagnose familial hypercholesterolaemia, the *LPA* KIV-2 repeat numbers (which are inversely associated with lipoprotein(a) concentrations) were lower in individuals with possible, probable, or definite familial hypercholesterolaemia than for those

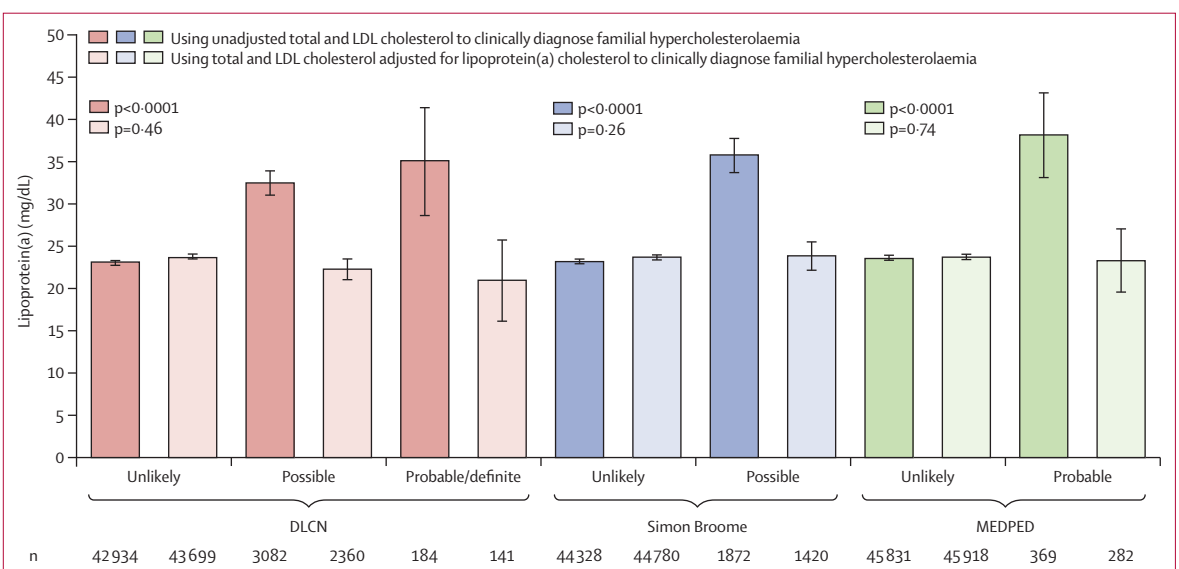

***Figure 1*: Mean concentrations of lipoprotein(a) by clinical familial hypercholesterolaemia status (based on DLCN, Simon Broome, and MEDPED criteria)**
Data are based on 46 200 white individuals from the Copenhagen General Population Study with lipoprotein(a) measurements. Error bars show 95% CIs. Information about familial hypercholesterolaemia mutation carrier status was not included during categorisation. DLCN=Dutch Lipid Clinic Network. MEDPED=Make Early Diagnosis to Prevent Early Death.

unlikely to have familial hypercholesterolaemia (p<0·0001; appendix p 11). These results were similar when the Simon Broome (p<0·0001) or MEDPED criteria (p=0·006) were used. Finally, when DLCN, Simon Broome, or MEDPED criteria were used to clinically diagnose familial hypercholesterolaemia, *LPA* rs10455872 single nucleotide carriers (which cause high lipoprotein(a) concentrations), were more common in individuals with possible (p=0·00012), probable (p≤0·0001), or definite (p=0·21) familial hypercholesterolaemia than in those unlikely to have familial hypercholesterolaemia (appendix p 11).

Mean lipoprotein(a) concentrations were 24 mg/dL (95% CI 23·4–24·0) in individuals without a familial hypercholesterolaemia mutation, 23 mg/dL(9–36) in individuals with an *LDLR* mutation (p=0·10 *vs* no known mutation), 21 mg/dL (14–28) in individuals with an *APOB* mutation (p=0·52), and 22 mg/dL (15–28) in individuals with any familial hypercholesterolaemia mutation (p=0·64; figure 2). By contrast, LDL cholesterol was raised in individuals with mutations causing familial hypercholesterolaemia.

We excluded 1111 individuals from the lipoprotein(a) analysis and 934 individuals from the KIV-2 analysis because they had an event of myocardial infarction before entry into the study. When DLCN criteria were used to clinically diagnose familial hypercholesterolaemia, the HRs for myocardial infarction compared with individuals unlikely to have familial hypercholesterolaemia and with lipoprotein(a) concentrations of 50 mg/dL or less were 1·4 (95% CI 1·1–1·7) in those with unlikely familial hypercholesterolaemia and lipoprotein(a) concentrations of more than 50 mg/dL, 3·2 (2·5–4·1) in those with possible, probable, or definite familial hypercholesterolaemia and lipoprotein(a) concentrations of 50 mg/dL or less, and 5·3 (3·6–7·6) in those with possible, probable, or definite familial hypercholesterolaemia and lipoprotein(a) concentrations of more than 50 mg/dL (figure 3). The corresponding HRs for myocardial infarction compared with individuals unlikely to have familial hypercholesterolaemia and with KIV-2 repeat numbers of more than 20% were 1·1 (95% CI 0·9–1·4) in those unlikely to have familial hypercholesterolaemia and with KIV-2 repeat numbers of 20% or less, 3·1 (2·4–4·0) in those with possible, probable, or definite familial hypercholestero—laemia and with KIV-2 repeat numbers of more than 20%, and 4·9 (3·4–7·1) in those with possible, probable, or definite familial hypercholesterolaemia and with KIV-2 repeat numbers of 20% or less (figure 3; HRs for covariates are shown in the appendix p 5). When we clinically diagnosed familial hypercholesterolaemia using DLCN, Simon Broome, or MEDPED criteria, the results were similar (appendix p 12). Also, when we used DLCN criteria and excluded lipoprotein(a) cholesterol content in total and LDL cholesterol to clinically diagnose familial hypercholesterolaemia, our results were similar overall, but with higher risk estimates for individuals with clinical familial hypercholesterolaemia and lipoprotein(a) concentrations of more than 50 mg/dL or KIV-2 repeat numbers of 20% or less (appendix p 13). Finally, when dividing the participants with clinical familial hypercholesterolaemia into those with and without clinical familial hypercholesterolaemia after excluding lipoprotein(a) cholesterol content in LDL cholesterol, the risk estimates for those with clinical familial hypercholesterolaemia after adjustment and with lipoprotein(a) concentrations of more than 50 mg/dL were also higher than for participants without clinical familial hypercholesterolaemia and lipoprotein(a) concentrations less than or equal to 50 mg/dL (appendix p 14).

Cumulative incidence of myocardial infarction as a function of age increased from individuals without clinical familial hypercholesterolaemia and lipoprotein(a) concentrations of 50 mg/dL or less, through those without clinical familial hypercholesterolaemia and lipoprotein(a) concentrations of more than 50mg/dL and those with possible, probable, or definite familial hypercholesterolaemia and lipoprotein(a) concentrations of 50 mg/dL or less, to those with possible, probable, or definite familial hypercholesterolaemia and lipoprotein(a) concentrations of more than 50 mg/dL (log-rank trend tests p<0·0001), based on DLCN criteria (figure 4). The

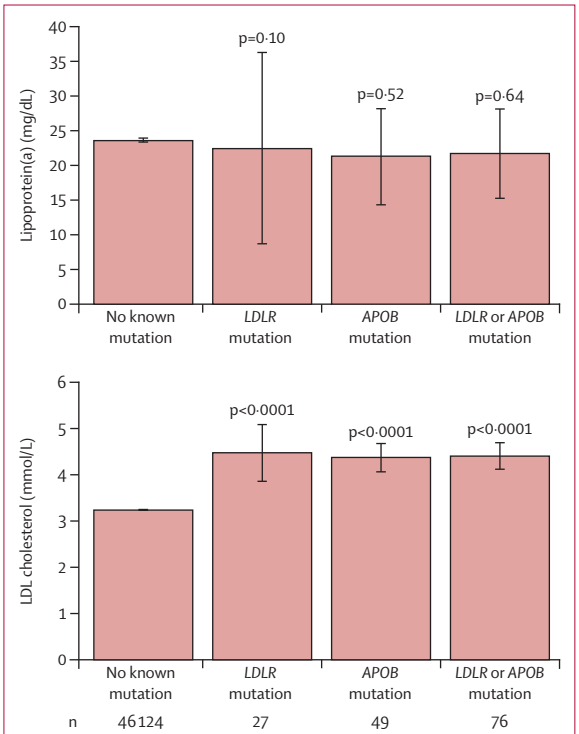

***Figure 2*: Mean concentrations of lipoprotein(a) and LDL cholesterol in individuals with and without a known familial hypercholesterolaemia mutation**

Data are based on 46 200 white individuals from the Copenhagen General Population Study with lipoprotein(a) measurements. Error bars show 95% CIs. The *LDLR* mutation category includes the Trp23X, Trp66Gly, and Trp556Ser mutations; *APOB* mutation includes the Arg3500Gln mutation only; *LDLR* or *APOB* mutation includes all four mutations. *LDLR*=low-density lipoprotein receptor. *APOB*=apolipoprotein B.

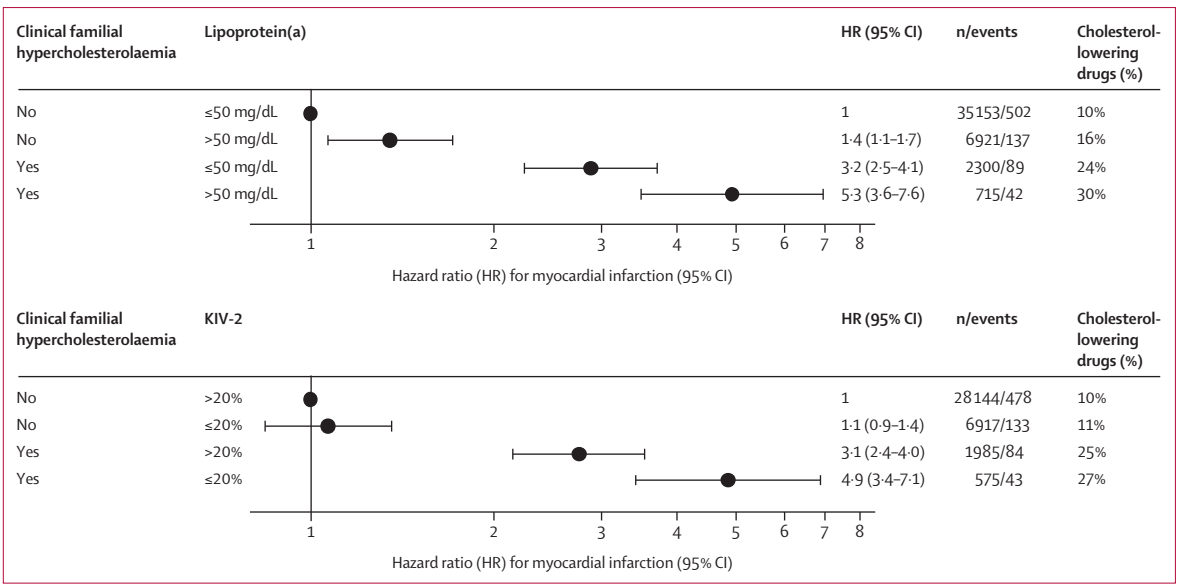

***Figure 3*: Risk of myocardial infarction as a function of clinical familial hypercholesterolaemia and lipoprotein(a) concentrations or *LPA* KIV-2 repeat numbers**
Data are based on 46 200 white individuals from the Copenhagen General Population Study included in the analysis with lipoprotein(a) measurements (top panel); of these, 38 555 incldued in the analysis also had *LPA* KIV-2 number of repeats measured (lower panel). Models were multifactorially adjusted for sex, age, smoking status, hypertension, diabetes, and use of cholesterol-lowering drugs. Diagnosis of clinical familial hypercholesterolaemia was based on the Dutch Lipid Clinic Network criteria, and the definition included participants with possible, probable, or definite familial hypercholesterolaemia. KIV-2=kringle IV type 2 number of repeats, expressed as percentiles of the distribution. FH=familial hypercholesterolaemia. HR=hazard ratio. n=number of participants. Events=myocardial infarction events.

corresponding cumulative incidences when stratifying for more than 20% and 20% or less KIV-2 repeat numbers were similar ($p<0·0001$; figure 4). When using DLCN, Simon Broome, or MEDPED criteria to clinically diagnose familial hypercholesterolaemia, the results were similar (appendix p 15). Also, when we used DLCN criteria excluding lipoprotein(a) cholesterol content in total and LDL cholesterol, results were similar overall, but with higher cumulative incidences for individuals with clinical familial hypercholesterolaemia and lipoprotein(a) concentrations of more than 50 mg/dL or KIV-2 repeat numbers of 20% or less than for individuals without clinical familial hypercholesterolaemia and lipoprotein(a) concentration less than or equal to 50 mg/dL or KIV-2 repeat numbers above 20% (appendix 16).

Discussion

In this prospective cohort study, we have shown that a 39–58% higher lipoprotein(a) concentration in individuals with clinical familial hypercholesterolaemia than in those unlikely to have familial hypercholesterolaemia was explained by lipoprotein(a) cholesterol contributing to LDL cholesterol in the clinical diagnosis of familial hypercholesterolaemia. Although it could be argued that this finding is not surprising, the fact that high lipoprotein(a) concentration has a potentially causal association with clinical familial hypercholesterolaemia has not, to our knowledge, previously been recognised or shown. Of all the individuals in this study with clinical familial hypercholesterolaemia, a quarter obtained the diagnosis because of high lipoprotein(a) concentrations, and high lipoprotein(a) in addition to high LDL cholesterol increased the already high risk of myocardial infarction in clinical familial hypercholesterolaemia.

Our data suggests that a quarter of all individuals with a clinical diagnosis of familial hypercholesterolaemia receive this diagnosis because of their high lipoprotein(a) concentrations. The cholesterol content of lipoprotein(a), estimated to be about 30% of lipoprotein(a) total mass,[15] was co-measured with total and LDL cholesterol (and included if LDL cholesterol was calculated with the Friedewald formula), and thus makes total and LDL cholesterol higher than when solely due to the LDL cholesterol increase caused by known familial hypercholesterolaemia mutations. Of the known mutations causing clinical familial hypercholesterolaemia, 95% are in the LDL receptor, 2–11% are in apolipoprotein B, and less than 1% are in PCSK9.[6,7] Our novel findings therefore suggest that variation in the *LPA* gene coding for apolipoprotein(a) in lipoprotein(a), and thus determining plasma lipoprotein(a) concentrations, could be the second most frequent cause of clinical familial hypercholesterolaemia.

It could be argued that raised lipoprotein(a) concentrations caused by *LPA* genetic variation is not a monogenic form of familial hypercholesterolaemia, like those caused by mutations in *LDLR*, *APOB*, and *PCSK9*, but more resembles polygenic familial hypercholesterolaemia.[21] However, even mutations in *LDLR*, *APOB*, and *PCSK9* will not always lead to clinical familial hypercholesterolaemia, and might only become clinically

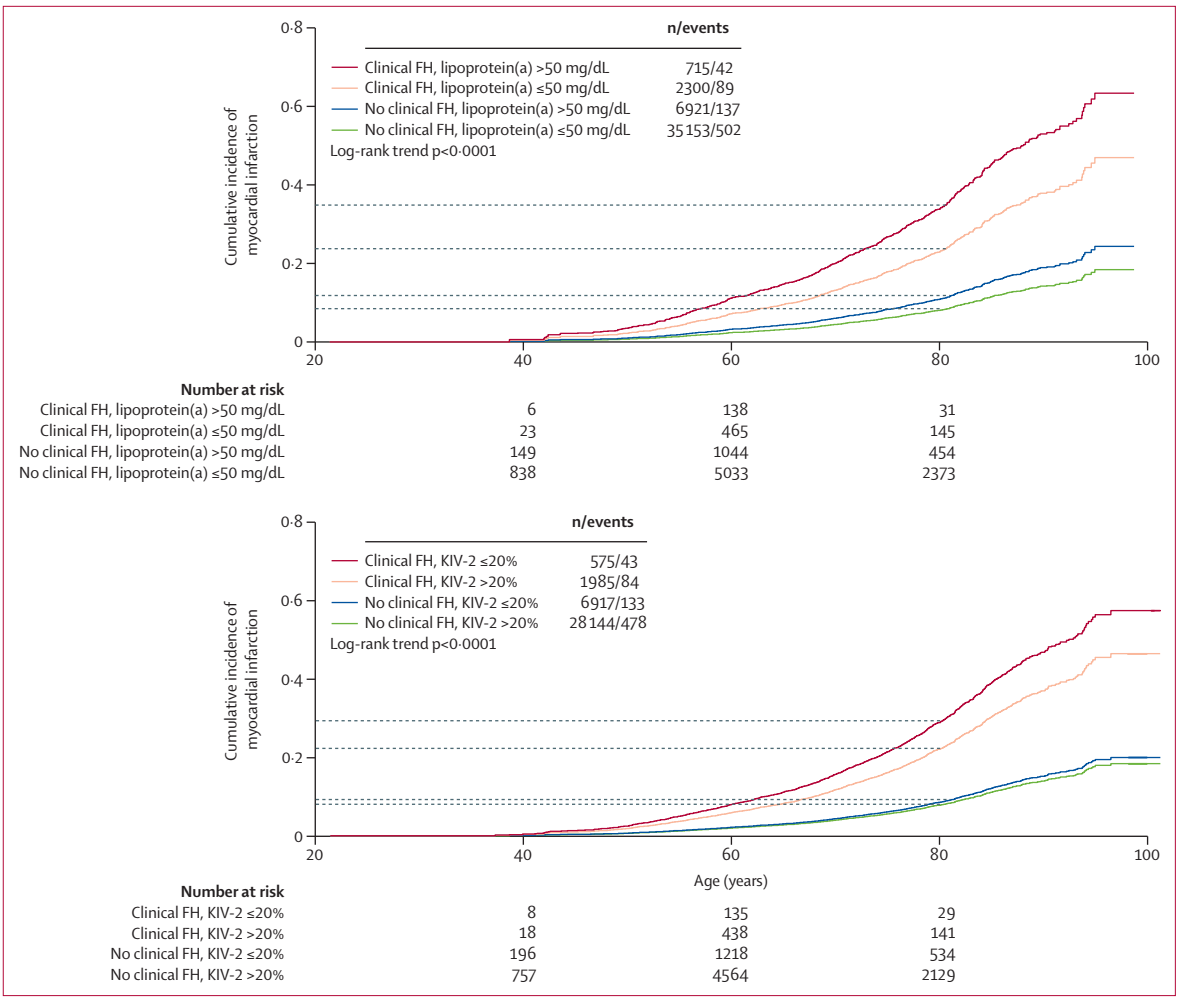

***Figure 4*: Cumulative incidences of myocardial infarction by age and as a function of clinical familial hypercholesterolaemia and lipoprotein(a) concentrations or *LPA* KIV-2 repeat numbers**

Data are based on 46 200 white individuals from the Copenhagen General Population Study with lipoprotein(a) measurements. Diagnosis of clinical familial hypercholesterolaemia was based on the Dutch Lipid Clinic Network criteria, and the definition included participants with possible, probable, or definite familial hypercholesterolaemia. The cumulative incidences by age 80 years are shown by dashed lines. KIV-2=kringle IV type 2 number of repeats, expressed as percentiles of the distribution.

apparent when lifestyle or other genetic factors contribute to raised LDL cholesterol concentrations. This scenario is similar to what our findings suggest for *LPA* genetic variation.

It could also be argued that raised lipoprotein(a) concentration is not a cause of clinical familial hypercholesterolaemia, but rather leads to misclassification of a different disease entitity as familial hypercholesterolaemia due to lipoprotein(a) cholesterol being included in LDL cholesterol for a clinical diagnosis of familial hypercholesterolaemia. However, this argument does not eliminate the fact that so far, LDL cholesterol has never had lipoprotein(a) cholesterol subtracted from it in common clinical practice, and therefore physicians worldwide will diagnose 25% of patients with clinical familial hypercholesterolaemia because of their high lipoprotein(a) concentrations.

Rather than contributing to LDL cholesterol concentrations and thus a diagnosis of clinical familial hypercholesterolaemia as shown in our study, the increase in lipoprotein(a) concentrations seen in familial hypercholesterolaemia might be speculated to be due to a reduced removal of lipoprotein(a) by the LDL receptor, because mutations in *LDLR* result in a reduced number of functional LDL receptors. However, statins, which cause an upregulation of the LDL receptor are unable to, or can only minimally, lower lipoprotein(a) concentrations.[22] Furthermore, data from human kinetic studies have suggested that lipoprotein(a) is not catabolised via the LDL receptor.[23] However, lipoprotein(a) concentrations have, in individuals with familial hypercholesterolaemia-causing mutations, been reported to be 12% higher than in relatives without familial hypercholesterolaemia;[24] notably, ascertainment bias might account for part of the higher

lipoprotein(a) concentrations reported, exactly like for LDL cholesterol concentrations.[25] Our findings therefore need be confirmed in cohorts in which all individuals have a diagnosis of familial hypercholesterolaemia due to a mutation (not polygenic) and the controls are relatives without familial hypercholesterolaemia, such as in the SAFEHEART Study.[24] Furthermore, in cell culture studies, lipoprotein(a) catabolism seems to be regulated by PCSK9 through the LDL receptor.[26] Despite these observations, the present findings of similar lipoprotein(a) concentrations in individuals with and without known familial hypercholesterolaemia mutations supports the notion that the LDL receptor is unlikely to remove lipoprotein(a) from the circulation under normal circumstances, and thus dysfunctional LDL receptors are unlikely to account for the high lipoprotein(a) concentrations seen in patients with clinical familial hypercholesterolaemia.

In support of the present findings, results from previous studies have shown that lipoprotein(a) concentrations are raised in individuals clinically diagnosed with heterozygous or homozygous familial hypercholesterolaemia.[6–9] Our data suggest that this finding is because of the high lipoprotein(a) concentrations per se and their co-measurement in total and LDL cholesterol, facilitating the clinical diagnosis of familial hypercholesterolaemia. Additionally, high lipoprotein(a) concentration as a heritable risk factor might contribute to personal and family history of premature cardiovascular disease, likewise facilitating diagnosis of clinical familial hypercholesterolaemia. Thus, individuals with high concentrations of lipoprotein(a) and familial hypercholesterolaemia-causing mutations probably present with more symptoms of cardiovascular disease, and are therefore more likely to be referred to a lipid clinic and receive the diagnosis of familial hypercholesterolaemia.

High lipoprotein(a) concentration is a common causal genetic risk factor for coronary heart disease and myocardial infarction in the general population.[1–5] Likewise, high lipoprotein(a) concentration is also a cardiovascular risk factor in patients with familial hypercholesterolaemia.[24,27–29] We have similarly shown that high concentrations of lipoprotein(a) add to the already high risk of myocardial infarction in individuals with familial hypercholesterolaemia in the general population. Our findings therefore lend support to the recommendation by the European Atherosclerosis Society of screening all patients with clinical familial hypercholesterolaemia for raised lipoprotein(a) concentrations.[4,6]

Cautious interpretation is important when comparing the 5·3-times risk of myocardial infarction in individuals with clinical familial hypercholesterolaemia unadjusted for lipoprotein(a) cholesterol content and lipoprotein(a) concentrations of 50 mg/dL or more with the corresponding value of 9·8-times risk when clinical familial hypercholesterolaemia was determined based on LDL cholesterol adjusted for lipoprotein(a) content. The most likely explanation for this difference is that when a clinical diagnosis of familial hypercholesterolaemia is determined after adjustment for lipoprotein(a) cholesterol, those individuals with clinical familial hypercholesterolaemia have the highest LDL cholesterol and therefore the highest risk. Or, put differently, when lipoprotein(a) cholesterol is not adjusted for, the lipoprotein(a) counts towards both determining the clinical diagnosis of familial hypercholesterolaemia and for the myocardial infarction risk due to lipoprotein(a) per se, and therefore the myocardial infarction risk is less than when the contributions of clinical familial hypercholesterolaemia and lipoprotein(a) are completely independent of each other.

Our study had several limitations. We did not have information about the children of participants or data about tendon xanthomas and arcus cornealis, so we could not include this information in our familial hypercholesterolaemia classification criteria, possibly resulting in a slight underestimation of clinical familial hypercholesterolaemia. Another limitation is that we only included adults of white Danish descent, so we do not know if our results are applicable to children and to individuals of other ethnic origins, particularly individuals of African descent, who tend to have higher lipoprotein(a) concentrations than white individuals;[4] however, we are not aware of any data to suggest that the present findings should not be applicable to children and to most ethnicities. We only included the most common familial hypercholesterolaemia mutations in Denmark, and therefore the group of participants with no known mutations might include a few individuals with other familial hypercholesterolaemia-causing mutations; however, the number of people in our study population with such mutations is probably very small, so this issue is unlikely to affect the results. Also, in our survival analysis we pooled individuals with possible and probable or definite familial hypercholesterolaemia (based on the DLCN criteria) to obtain maximum statistical power, even though not all of these individuals will have clinical familial hypercholesterolaemia. Furthermore, LDL cholesterol concentrations are adjusted by 43% to reflect patients receiving cholesterol-lowering drugs based on average dosed effects, which is probably a simplification of the real effect with varying doses and individual differences. Additionally, index case criteria such as the DLCN, Simon Broome, and MEDPED used in this study could be regarded inappropriate to use in family screening for familial hypercholesterolaemia; however, we used these criteria in a general population study. Another limitation is that in most individuals, LDL cholesterol was calculated in non-fasting samples; however, in the Copenhagen City Heart Study, we previously measured LDL cholesterol directly as well as calculating it from the Friedewald equation in 5436 individuals using non-fasting samples with similar results (R^2=0·84; measured LDL cholesterol=0·95 multiplied by calculated LDL cholesterol

plus 0·29). Finally, direct methods exist that can differentiate lipoprotein(a) from LDL cholesterol and measure absolute concentrations of cholesterol in each particle fraction, as does a more accurate method to measure LDL cholesterol (magnetic resonance).

Our results suggest that all individuals with clinical familial hypercholesterolaemia should have lipoprotein(a) concentrations measured in order to identify those with the highest concentrations and, as a result, the highest risk of myocardial infarction. Also, if during genetic testing, a familial hypercholesterolaemia-causing mutation is not found in *LDLR*, *APOB*, or *PCSK9*, it might be that variation in the *LPA* gene is a major contributing cause of clinical familial hypercholesterolaemia. Ideally, both LDL cholesterol corrected for lipoprotein(a) and uncorrected should be reported for familial hypercholesterolaemia diagnosis, so that the quarter of patients with clinical familial hypercholesterolaemia caused by high lipoprotein(a) can be separated out and excluded from genetic testing; however, this approach does not seem realistic in most clinical settings. Furthermore, at present, the main treatment for individuals with familial hypercholesterolaemia is highly potent statin treatment, and if the main driver of some cases of clinical familial hypercholesterolaemia is high lipoprotein(a) concentration then the effect of statin treatment might be limited. With the development of novel lipoprotein(a)-lowering treatments,[11,12,30] it might be relevant to distinguish between clinical familial hypercholesterolaemia mainly caused by raised LDL cholesterol from that mainly caused by raised lipoprotein(a). Such differentiation would also allow the identification of individuals for whom family screening for lipoprotein(a) levels could be done, to identify family members at increased cardiovascular risk.

Contributors

AL and BGN designed the study, did the analyses, interpreted the findings, and wrote and revised the report. PRK, MB, and AT-H contributed to data collection, data preparation, or both. All authors contributed to critical reading and revision of the draft report.

Declaration of interests

PRK has received lecture or consultancy honoraria from Fresenius, Sanofi, and Regeneron. AT-H has received lecture or consultancy honoraria from Lilly and LGC Genomics. BGN has received lecture or consultancy honoraria from AstraZeneca, Merck, Omthera, Sanofi, Regeneron, Ionis Pharmaceuticals, Aegerion, Dezima, Fresenius, B Braun, Kaneka, Pfizer, Amgen, Lilly, and Denka Seiken. AL and MB declare no competing interests.

Acknowledgments

This study was supported by the Danish Heart Association and IMK General Fund, Copenhagen, Denmark. We thank the staff and participants of the Copenhagen General Population Study for their important contributions, and to the laboratory technicians Anja Jochumsen, Anders Reuss, Trine Søberg Nielsen, and Anitta Pedersen for their excellent work.

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